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(54) Title: METHOD FOR DOSING THERAPEUTIC COMPOUNDS

(57) Abstract

The internally cyclized congeners of hydroxy-substituted nucleotide analogues have been found to exhibit substantially lower toxicity *in vivo* than their uncyclized analogues, while retaining essentially the same antiviral activity. This was unexpected because the prior art would have suggested that the cyclic analogues offered no significant advantages in respect to toxicity *in vivo*. This finding permits the administration of much greater doses of the cyclic congeners than otherwise would have been possible and/or allows the clinician to omit toxicity ameliorating interventions. Novel compounds are provided for use in the method of this invention. Novel methods for the preparation of these compounds also are provided.

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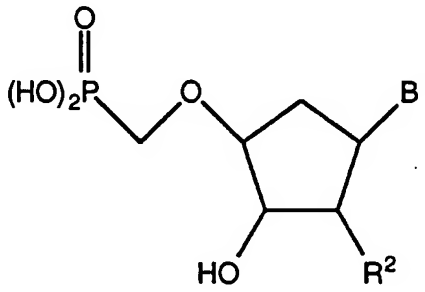
METHOD FOR DOSING THERAPEUTIC COMPOUNDS

This invention relates to methods and compounds for the treatment of viral infections, including prophylaxis. In particular it is concerned with the management of kidney toxicity by selection of therapeutic dosages of antiviral compounds.

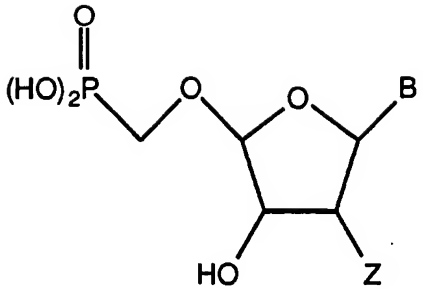
A number of antiviral compounds are known that are characterized by a phosphonate group linked to a nucleotide base via a hydroxy-substituted cyclic or acyclic linking moiety, wherein the hydroxy group is joined by 4 atoms (typically as alkyl or alkoxyalkyl chains) to the phosphorus atom and the phosphorus atom is bonded to a methylene group of the cyclic or acyclic linking moiety. These hydroxy-substituted nucleotide analogues (herein, "HSNAs") include the compounds of structures (I) - (VII) below. Structure (I) compounds are disclosed in EP 369,409 and/or U.S.S.N. 08/110,841 (pending):



(I)

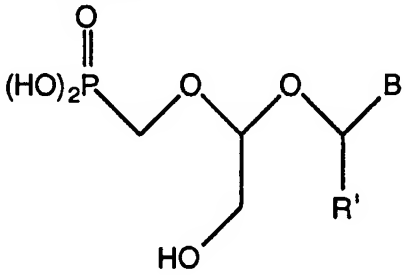
wherein B is a heterocyclic group having at least 1 nitrogen heteroatom and up to 3 additional heteroatoms selected from nitrogen, oxygen and sulfur, said heterocyclic group being connected through a nitrogen heteroatom thereof, and R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino, or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino.

Structure (II) and (III) compounds are disclosed in EP 398,231:



(II)

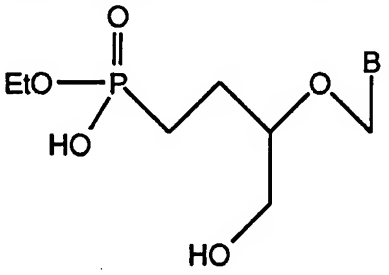
wherein Z is hydrogen or C_1-C_6 alkyl and B is a 9-substituted purine or 1-substituted pyrimidine base; and



(III)

wherein R' is hydrogen, C_1-C_6 alkyl or hydroxyalkyl with 1-6 carbon atoms, and B is a 9-substituted purine or 1-substituted pyrimidine base.

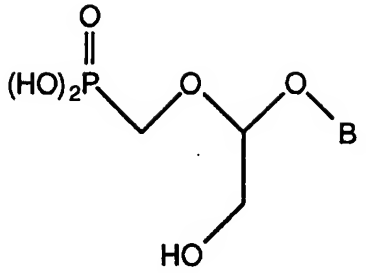
Barnard et al. ("Antiviral Research" 22:77-89 [1993]; see also WO 94/03466) disclose HSNA compounds of structure (IV):



(IV)

wherein B is guanin-9-yl.

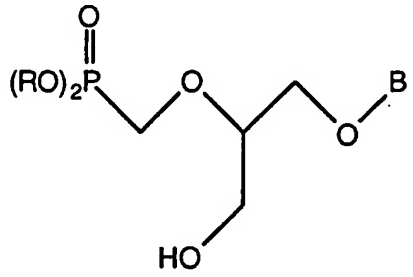
U.S. Patent No. 5,208,221 discloses HSNA compounds of structure (V):



(V)

wherein B is a 9-substituted purine or 1-substituted pyrimidine base.

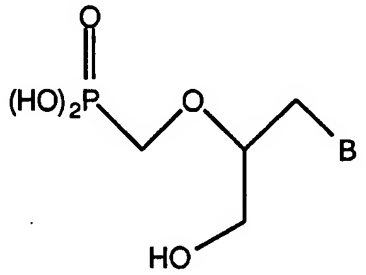
U.S. Patent No. 5,247,085 discloses HSNA compounds of structure (VI):



(VI)

wherein R is H, C_1-C_6 alkyl, or optionally substituted phenyl and B is one of a group of defined purin-9-yl bases.

Other well-known HSNAs include certain 3-hydroxy-2-(phosphonomethoxy)propyl analogues of nucleotide bases (herein, "HPMPB") of structure (VII):

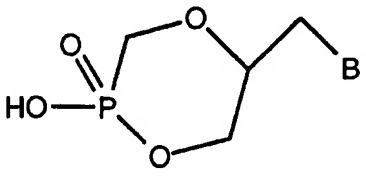


(VII)

wherein B is a pyrimidin-1-yl, pyrimidin-3-yl, purin-3-yl, purin-7-yl or purin-9-yl residue, or the deaza, aza or deaza-aza analogues thereof. These compounds are active against DNA viruses. The principal members of the HPMP class are the compounds of structure (VII) in which B is cytosin-1-yl

(herein, "HPMPC") or adenin-9-yl (herein, "HPMPA"). The (S) isomers are preferred. See U.S. Patent Nos. 5,142,051 and 4,724,233.

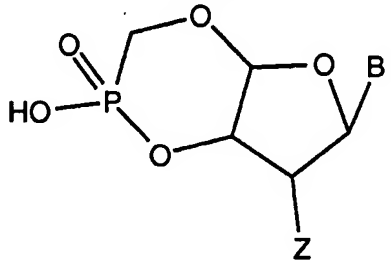
It is known to internally cyclize certain HSNA compounds. cHPMPBs are the internally cyclized congeners of the corresponding HPMPB and have structure (VIIa):



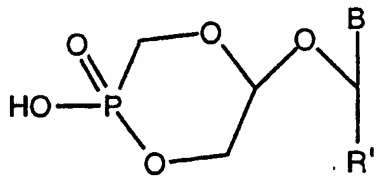
(VIIa)

wherein B is as defined in structure (VII). Two examples of the compounds of structure (VIIa) are known: cHPMPA and cHPMPC (U.S. Patent No. 4,724,233, Andrei et. al., "Eur. J. Clin. Microbiol. Infect. Dis.", 10(12):1026-1033 [1991]; Lin et. al., "Antimicrobial Agents and Chemotherapy", 35(11):2440-2443 [1991]; Snoeck et. al., "Antiviral Research" 16:1-9 [1991]; Andrei et. al., "Antiviral Research", 20(Suppl. 1):109 [1993]; Ho et. al., "Mol. Pharmacology" 41:197-202, [1992]). The (S) enantiomer of cHPMPC bears the IUPAC name 1-[((S)-2-hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl] cytosine (CAS Reg. No. 127757-45-3).

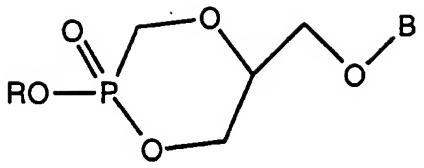
In addition, the internally cyclized analogues of the compounds of structures (II) and (III) (EP 398,231) and (VI) (U.S. Patent No. 5,247,085) are known. They have the structures (IIa), (IIIa) and (VIa):



(IIa)



(IIIa)



(VIa)

wherein Z, R, R' and B are defined above in structures (II), (III) and (VI).

HPMPC has been extensively studied and currently is in human clinical trials. Its cyclic congener has received comparatively little attention. However, cHPMPC has been reported to have activity against cytomegalovirus in human embryonic lung cells *in vitro* (Snoeck et al., "Antiviral Research" 16:1-9 [1991]). The Snoeck et al. data suggest that cHPMPC is less toxic than HPMPC, but less efficacious as well by approximately the same degree. In particular, Snoeck et al. reported that the micromolar cytotoxicities of cHPMPC and HPMPC were 720 and 360, respectively, by cell growth and 108 and 72, respectively, by radiothymidine incorporation. Holy et al. ("Antiviral Research" 13:295-312 [1990]) reported similar *in vitro* cell culture data. See also Snoeck et al., "Int. Congr. Ser.-Excerpta Med., 978 (Prog. Cytomegalovirus Res.) 337-340 (1991) and Holy et al., "Coll. Czech. Chem. Commun." 54(a): 2470-2501 (1989).

Li et al. have reported that HPMPC is nephrotoxic in guinea pigs (see "Antiviral Research" 13:237-252 [1990]), and nephrotoxicity is the limiting toxicity in human clinical trials of HPMPC. Human nephrotoxicity is ameliorated by concomitant administration of probenecid and by giving fluids prior to HPMPC administration (hydration). In contrast to the extensive studies of HPMPC, the published literature is believed to be devoid of any animal studies of efficacy or toxicity of cHPMPC.

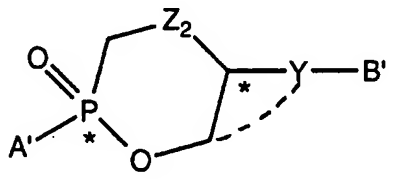
It is an object of this invention to enlarge the therapeutic window for HSNAs by supplying them in a form that is less toxic *in vivo* while substantially retaining the antiviral activity of the HSNA. In addition, it is an object of the invention to reduce or eliminate the practice of hydration or probenecid administration during a course of HPMPC therapy and to minimize the need to withdraw patients from HSNA treatment due to the development of kidney toxicity. An additional object of this invention is to facilitate non toxic increases in the dose, frequency and length of

administration of HSNAs. It is a further object of this invention to provide novel internally cyclized derivatives of certain HSNAs. Another object of this invention is to provide intermediate forms of cHSNAs having oral bioavailability, reduced toxicity and greater efficacy, together with novel methods for their manufacture.

Summary of the Invention

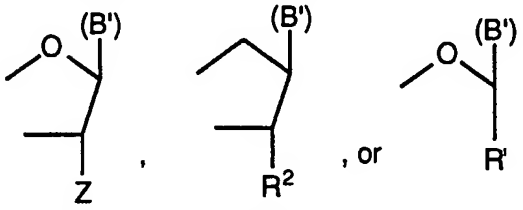
The objects of this invention are accomplished by administering to a subject an antivirally effective, non-cytotoxic dose of cHSNA which is in excess of the maximum non-cytotoxic dose for the corresponding uncyclized HSNA. In certain embodiments the maximum non-cytotoxic dose is defined in terms of the maximum non-nephrotoxic dose. In preferred embodiments, particularly where the HSNA is HPMPC, the cHSNA dose is in excess of 2 times the HSNA maximum non-cytotoxic dose. In additional embodiments an antivirally effective course of therapy of cHPMPC or other cHSNA is administered without probenecid and/or hydration.

The cHSNAs to be used in the practice of this invention have structure (VIII)



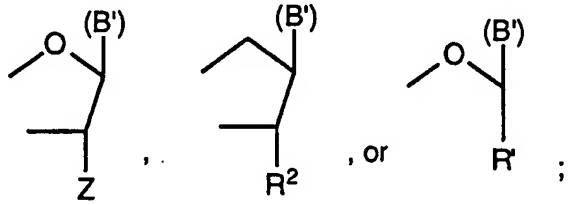
(VIII)

wherein Z_2 is oxygen or methylene, Y is $-CH_2-$, $-OCH_2-$, -O-

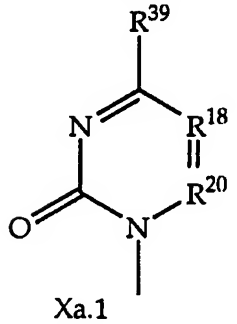


Z, R', and R^2 are defined above, A' is OH or A, A is an amidate or ester, the stereochemistry of the carbon and phosphorus atoms denoted with the asterisks independently is (S), (R) or (RS), the orientation of the Y groups is shown by (B'), and B' is a heterocyclic base.

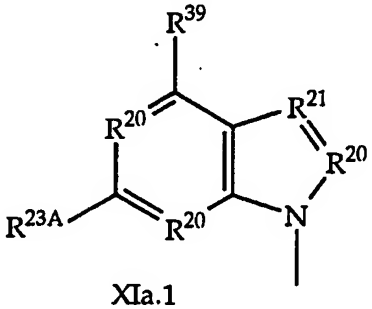
In another embodiment a novel intermediate is provided having structure (VIII) wherein A' is hydroxyl, an amidate or an ester, Z_2 is oxygen or methylene, Y is $-OCH_2-$, -O-



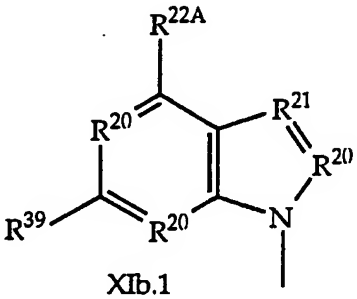
the stereochemistry of the phosphorus and carbon atoms designated by asterisks independently is R, S or RS, the orientation of the Y groups is designated by (B') and B' is a base of the formula (Xa.1), (XIa.1) or (XIb.1)



Xa.1



XIa.1



XIb.1

wherein R^{18} is N, CF, CCl, CBr, CI, CR^{19} or CSR^{19}, COR^{19};

R^{19} is H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2 - C_9 alkynyl, C_1-C_9 alkyl-C_1-C_9 alkoxy, or C_7-C_9 aryl-alkyl unsubstituted or substituted by OH, F, Cl, Br or I including CH_3, CH_2CH_3, $-CHCH_2$, $-CHCHBr$, CH_2CH_2Cl, CH_2CH_2F, $-CH_2CCH$, $-CH_2CHCH_2$, C_3H_7, CH_2OH, CH_2OCH_3, $CH_2OC_2H_5$, $-CH_2OCCH$, $-CH_2OCH_2CHCH_2$, $CH_2C_3H_7$, CH_2CH_2OH, $CH_2CH_2OCH_3$, $CH_2CH_2OC_2H_5$, $-CH_2CH_2OCCH$, $-CH_2CH_2OCH_2CHCH_2$, $CH_2CH_2OC_3H_7$;

R^{20} is N or CH;

R^{21} is N, CH, CCN, CCF_3, $CC{\equiv}CH$ or $CC(O)NH_2$;

R^{22A} is R^{39} or R^{22}, provided that R^{22} is not amino;

R^{23A} is R^{39} or R^{23}, provided that R^{23} is not amino;

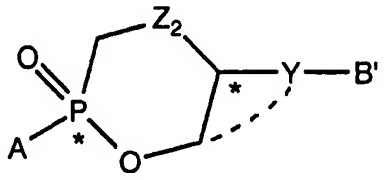
R^{39} is NHR^{40}, $NHC(O)R^{36}$ or $CR^{41}N(R^{38})_2$ wherein R^{36} is C_1-C_{19} alkyl, C_1-C_{19} alkenyl, C_3-C_{10} aryl, adamantoyl, alkylaryl, or C_3-C_{10} aryl substituted with 1 or 2 atoms or groups selected from halogen, methyl, ethyl, methoxy, ethoxy, hydroxy and cyano;

R^{38} is C_1-C_{10} alkyl, or both R^{38} together are 1-morpholino, 1-piperidine or 1-pyrrolidine;

R^{40} is C_1-C_{20} alkyl; and

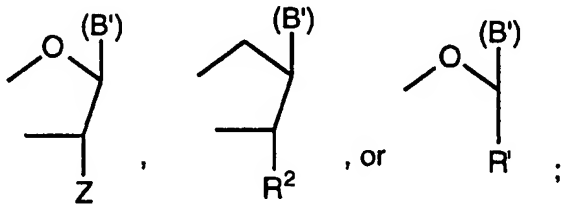
R^{41} is H or CH_3; and salts thereof.

In another embodiment a novel intermediate is provided having structure (VIIIa)



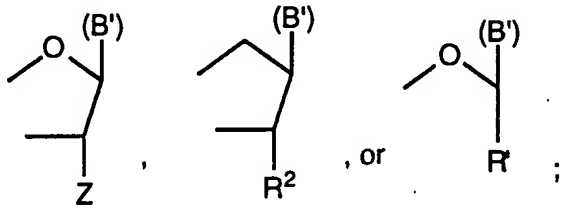
(VIIIa)

wherein A is an amidate or an ester, Z_2 is oxygen or methylene, Y is $-OCH_2-$, -O-



Z is H or C_1-C_6 alkyl; R' is hydrogen, C_1-C_6 alkyl or hydroxyalkyl with 1-6 carbon atoms; R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino; the stereochemistry of the carbon and phosphorus atoms denoted with the asterisks independently is (S), (R) or (RS), the orientation of the Y groups is shown by (B'), and B' is a heterocyclic base provided, however, that (a) A is not a C_1-C_6 alkyl ester or, (b) when Z_2 is oxygen and Y is $-OCH_2-$ then A is not phenyl or substituted phenyl; and the salts thereof.

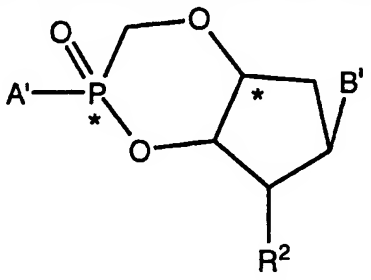
In another embodiment a novel intermediate is provided that has the structure (VIIIa) wherein A is an amidate or an ester; Z_2 is oxygen or methylene; Y is $-OCH_2-$, -O-



Z is H or C_1-C_6 alkyl; R' is hydrogen, C_1-C_6 alkyl or hydroxyalkyl with 1-6 carbon atoms; R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino; the orientation of

the Y groups is shown by (B'), B' is a heterocyclic base; the stereochemistry of the carbon atom designated by an asterisk is R, S or RS; the intermediate is enriched or resolved at the phosphorus atom chiral center; and the salts thereof.

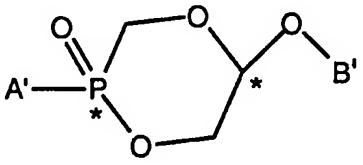
In other embodiments of the invention, novel compounds are provided which have the structures (Ia) and (Va):



(Ia)

wherein * designates (S), (R) or (RS) configuration, and B', R^2 and A' are defined above, together with the salts thereof.

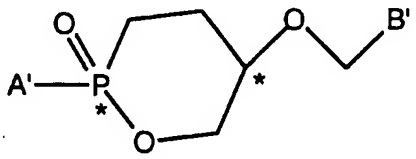
Structure (Va) is



(Va)

wherein * and A' and B' are defined above, together with the salts of such compounds.

Also useful in the method of this invention are compounds of structure (IVa)



(IVa)

wherein * and A' and B' are defined above, together with the salts of such compounds.

In each of (Ia) and (Va), the carbon atom * chiral center preferably is (S); in (IVa) it preferably is (R).

Detailed Description of the Invention

HPMPC (cidofovir) is the HSNA for which the greatest human clinical experience is available. Administration of cidofovir to HIV-infected patients with asymptomatic CMV infection of urine and semen has been associated with dose-dependent nephrotoxicity and a dose-dependent anti-CMV effect.

Initial multiple dose administration of cidofovir was performed with the objectives of identifying the safety, pharmacokinetics, and anti-CMV effects of twice weekly or weekly intravenous infusions of cidofovir with or without concomitant saline prehydration (Table 1).

Table 1
Phase I/II Cidofovir Regimens

Dose (mg/kg)	Schedule	Hydration[1]
0.5, 1.5, 5.0	biw	+/-
0.5, 1.0, 3.0, 10.0	q week	+/-

[1]Administered as one liter normal saline over approximately 45 min immediately prior to cidofovir infusion.

Patients received cidofovir during 4 consecutive weeks. Patients completing the 4 week study without evidence of drug-related toxicity were offered continuing weekly cidofovir maintenance therapy.

Patients tolerated doses of 0.5, 1.0, or 1.5 mg/kg without evidence of significant drug-related clinical or laboratory toxicity. Patients treated at the highest dose levels (5.0 mg/kg twice weekly and 3.0 or 10.0 mg/kg once weekly) developed evidence of cidofovir-related nephrotoxicity manifested by proteinuria, glycosuria, and decreases in serum phosphate, uric acid, and bicarbonate consistent with renal proximal tubular cell injury, as predicted by preclinical animal studies. Two of 5 patients receiving 3.0 mg/kg once weekly developed Grade II nephrotoxicity (serum creatinine of $\geq$ 2.0 mg/dL or 2+ proteinuria) following 6 and 14 doses of cidofovir, respectively. The first patient's course was complicated by intercurrent adrenal insufficiency and severe volume depletion. Two of 5 patients receiving 10.0 mg/kg once weekly of cidofovir developed evidence of persistent Grade IV nephrotoxicity following two doses. Both patients had evidence of non-

oliguric renal insufficiency consistent with proximal tubular cell injury. Each of the above 4 patients displaying nephrotoxicity did not receive concomitant hydration during the antecedent cidofovir infusions. Persistent nephrotoxicity (≥ Grade II) was not observed in patients receiving concomitant hydration with cidofovir.

Proteinuria, as measured by routine urinalysis, is an early indicator of cidofovir-related nephrotoxicity. Interruption of cidofovir treatment following the appearance of proteinuria or an absolute serum creatinine increase of 0.5 mg/dl permitted the administration of systemic cidofovir without significant drug-related toxicity.

A dose-dependent anti-CMV effect was observed at dose ≥ 3.0 mg/kg. Reduction in semen CMV titers of greater than 100-fold and conversion of positive urine CMV cultures to negative occurred in a majority of patients treated at these dose levels. These effects were seen as early as one week following the administration of 10.0 mg/kg of cidofovir as a single dose. Additionally, serial semen cultures documented persistent anti-CMV effect following discontinuation of cidofovir; culture negativity persisted for approximately 30 days following cessation of treatment at the 10 mg/kg dose level.

Identification of the sequence of urinalysis and serum chemistry abnormalities associated with cidofovir-related nephrotoxicity, as well as demonstration of prolonged anti-CMV effect, has led to modifications in the methods of cidofovir administration (Table 2). For example, interruption of cidofovir treatment following the appearance of ≥ 1+ proteinuria or an absolute increase in serum creatinine of 0.5 mg/dl permitted the administration of systemic cidofovir without significant drug-related toxicity. In addition, investigation of longer dosing intervals (1, 2, and 3 weeks) was pursued. Additionally, as suggested by preclinical animal studies, concomitant administration of probenecid was employed in an effort to block uptake of cidofovir by the proximal tubular cell of the kidney.

Table 2
Phase I/II Cidofovir Regimens:
Dose - Refinements

Dose (mg/kg)	Schedule	Hydration[1]
3.0 + Probenecid[2]	q week	+/-
5.0 + Probenecid	q week	+/-
5.0 + Probenecid	q 2 weeks	+/-
7.5 + Probenecid	q 3 weeks	+/-

[1]Administered as one liter normal saline over approximately 45 minutes immediately prior to cidofovir infusion.

[2]Administered orally as 2 grams (3 h pre-cidofovir), 1 gram (2 h post-cidofovir), and 1 gram (8 h post-cidofovir) (total dose = 4 grams).

Thirty-two patients received cidofovir with concomitant probenecid (8 patients at 3 mg/kg [range 10-20 doses]; 18 patients at 5 mg/kg [range 2-13 doses]; and 6 patients at 7.5 mg/kg [range 1-8 doses]). Five of the 32 patients developed ≥ 2+ proteinuria, but none developed significant creatinine elevation.

Preliminary evidence of a dose-dependent anti-CMV effect was observed in patients receiving cidofovir in combination with probenecid. Comparison of decreases in semen titer of CMV after 4 consecutive weekly doses of cidofovir (3 mg/kg) +/- probenecid suggested enhanced anti-CMV effect in patients receiving concomitant probenecid.

Determination of Maximum Non-Cytotoxic Dosages of HSNA's; cHSNA Dosing

The term "maximum non-cytotoxic dose" (hereafter "MND") means the maximum molar quantitative amount of HSNA that can be administered to the subject in question without inducing a toxic response that, in the opinion of the ordinary reasonable clinician, would necessitate a reduction in dose of the HSNA or the withdrawal of the subject from treatment with the HSNA. The MND for a given subject will vary depending on a number of factors, including the pre-existing condition of the patient, (the MND will be lower if the patient already is demonstrating injury to an organ for which the HSNA is cytotoxic), the nature of the

cytotoxicity (potentially life-threatening cytotoxicities, e.g. for organs such as kidney or liver, will lower the MND), the frequency of administration of the HSNA (giving the same dose of HSNA in dispersed doses as opposed to a bolus generally will lower the MND for the HSNA over a given period of time, e.g., 50 mg/kg as a single dose in African green monkeys is much less toxic than 5 mg/kg/d for 10 days), the period that the subject has been on the HSNA (longer periods of therapy on HSNA generally will lower the MND for subsequent dosings), the species concerned (the order of sensitivity to HPMPC is guinea pig > rabbit > monkey > rodent), the presence or absence of concomitant therapies that may exacerbate or to ameliorate the expected cytotoxicity, and possibly the administration route (subcutaneous masses were observed with SC administration of HPMPC in a 26-week toxicology study in Sprague-Dawley rats, but not in a 3-month intravenous HPMPC study in Sprague-Dawley rats at doses up to 100 times greater than the lowest SC dose). It is possible with minimal experimentation to determine the MND for the ordinary subject, for example patients not bearing any unusual pre-existing conditions and not requiring coadministration of agents expected to exacerbate the HSNA cytotoxicity in question. This MND can be used to establish the initial dose for subsequent patients in the same cohort. In any case, the practice per se of monitoring and optimizing therapeutic dosing even in individual patients is a long standing and conventional practice, and it would not require any experimental effort outside that which is ordinarily undertaken by the clinician.

Frequently encountered HSNA cytotoxicities include skin irritation (when administered topically), punctal stenosis (when administered by opthalmic modes of delivery, such as eyedrops) and nephrotoxicity by systemic treatment as described above. cHSNAs are expected to exhibit substantially less of these cytotoxicities while still having essentially the same antiviral activity, thereby permitting the molar dose of cHSNA to exceed the MND of the corresponding HSNA. Since the *in vivo* antiviral activity of the cHSNA is essentially the same as the HSNA on an equimolar dose basis, administration of the cHSNA dose above the MND of the HSNA will greatly increase therapeutic antiviral activity.

Nephrotoxicity is the dose-limiting toxicity for many HSNAs, and is the current barrier to administration of larger doses of HPMPC by systemic

routes. Accordingly, the MND for systemically-administered HPMPC is equivalent to its maximum non-nephrotoxic dose.

The term "non-nephrotoxic dose" means a systemic dose administered by a route, frequency and amount that fails to produce a clinically relevant loss of renal function. In some patients a dose that fails to produce 2+ proteinuria as measured by urinalysis reagent strips will be considered non-nephrotoxic. Alternatively, (and preferably for patients with pre-existing kidney damage) the non-nephrotoxic dose is a dose that fails to produce nephrotoxicity within about 14 days after the preceding dose, nephrotoxicity being indicated by a substantial percentage loss in renal function as conveniently measured by serum creatinine changes. Typically, an increase of >0.5 mg/dl of serum creatinine over the subject's baseline prior to the preceding dose will constitute a threshold indication of nephrotoxicity. It is desirable to use the patient's baseline because serum creatinine is subject to some variation, on the order of ± 0.2 mg/dl, resulting from factors other than renal integrity. The "maximum non-nephrotoxic dose" means the greatest amount of HSNA by a given route and frequency that can be administered to a subject without producing 2+ proteinuria or >0.5 mg/dl increase in creatinine as noted. The MND for HPMPC has been established in primates and man. In addition to the foregoing clinical studies there are the following:

The minimal lethal IV single dose of cidofovir in cynomolgus monkeys is estimated to be greater than 40 mg/kg and less than 75 mg/kg. Mortality in one monkey at the 75 mg/kg dose level was preceded by evidence of nephrotoxicity (elevated BUN and creatinine) and decreased white blood cell count. In contrast, administration of 50 mg/kg as a single IV dose to African green monkeys was well-tolerated without histologic evidence of nephrotoxicity.

Dose-ranging IV studies performed in cynomolgus monkeys receiving 0.1 to 50 mg/kg/day or 0.1 to 1.0 mg/kg/day for 14 or 30 days, respectively, also revealed evidence of dose-related nephrotoxicity. Significant drug-related clinical or histologic toxicity was not observed at 1 or 0.25 mg/kg/day (over 14 or 30 days, respectively).

In two 13-week subchronic IV toxicity studies in cynomolgus monkeys, cidofovir administered once weekly resulted in nephropathy at 5 mg/kg/week, and in testicular degeneration at 2 mg/kg/week. A NOEL of 1 mg/kg/week was observed for both kidney and testes changes. Co-

treatment with orally-administered probenecid minimized the severity of cidofovir-mediated nephrotoxicity, but did not affect the severity of the testes changes.

Cidofovir was administered to African green monkeys to further investigate schedule dependency as well as reversibility of toxicity. As observed in guinea pig experiments, a single IV dose of 50 mg/kg was not associated with evidence of nephrotoxicity in contrast to animals receiving 10 daily IV doses of 5 mg/kg/day.

Finally, dose-ranging oral administration studies have been performed. Cynomolgus monkeys received 1, 5, or 25 mg/kg of cidofovir by oral gavage, twice weekly for five doses. Significant drug-related clinical toxicity was only observed in the high dose group; all such animals had evidence of gastrointestinal toxicity including diarrhea or constipation. One animal receiving 25 mg/kg expired 24 h after the final cidofovir dose. Histologic examination of all treated animals revealed evidence of significant enteropathy (large and small intestine) in the high dose group. Additionally, renal morphologic abnormalities, including tubular cell hypertrophy and necrosis, were observed in the high dose group but not in the lower dose groups.

The patient being treated may be exposed to nephrotoxic agents (e.g. cyclosporine or amphotericin B) or have pre-existing kidney damage, in which case the maximum non-nephrotoxic dose in these patients will be lower than in most patients. On the contrary, supplemental therapies directed at ameliorating nephrotoxicity will cause the maximum non-nephrotoxic dose to be higher than in patients not receiving such therapies, e.g., administration of probenecid or other agents for inhibiting the uptake of anions by the kidney, or adequately hydrating patients by infusion or oral intake of aqueous solutions, will raise the maximum non-nephrotoxic dose. Accordingly, the range of non-nephrotoxic doses will vary somewhat from patient-to-patient depending upon these and other factors known to the artisan. In general, one must take into account the condition of the patient, the distribution of the dosage over time, the amount of time the patient has been on drug, the administration route, the frequency of administration, the animal species being treated, the use of nephroprotective measures such as probenecid and hydration, and the concomitant administration of nephrotoxins.

A salient feature of this invention is that substantially the same antiviral efficacy of an HSNA such as HPMPC can be achieved with the same dosage of cyclic analogue of the HSNA, but with much less toxicity, in particular nephrotoxicity. This means that the minimum non-nephrotoxic, antivirally active dose of the cHSNA will be greater than the maximum non-nephrotoxic dose of the HSNA on a molar basis, all other therapeutic influences being essentially the same as noted above.

The lower toxicity of cHPMPC compared with that of HPMPC in rats is demonstrated in the examples in two studies. In Example 1, a direct comparison of HPMPC at 100 mg/kg with cHPMPC at 250 mg/kg showed kidney effects with the former but not the latter. Therefore, cHPMPC has an improved safety margin of at least 2.5-fold. In Example 2, a comparison of results with those from a nonconcurrent study of HPMPC in rats showed that the nephrotoxic effects caused by 3 mg of HPMPC per kg are equal to or greater than the effects caused by 40 mg of cHPMPC per kg. Thus, cHPMPC has an apparent improved safety margin of ≥13-fold in rats. In addition, preliminary findings in 1-month studies in rats and monkeys show that at least a 10-fold dose increase of cHPMPC is needed to produce nephrotoxicity equivalent to that of HPMPC.

Since cHPMPC is substantially less toxic, but similarly efficacious compared to HPMPC, one can employ substantially greater systemic molar doses of cHPMPC than the maximum non-nephrotoxic dose of HPMPC and still not induce nephrotoxicity in patients. In most embodiments, the typical cHPMPC molar dosage will be greater than twice (on a molar basis) the HPMPC maximum non-nephrotoxic or non-cytotoxic dose, although it also may be 3, 4, 5, 6, 7, 8, 9 or 10 times the maximum non-nephrotoxic or non-cytotoxic dose, or it will be the same as a conventional HSNA dose but without the use of probenecid and, optionally, hydration.

The maximum non-nephrotoxic single weekly dose for HPMPC for humans in the ordinary clinical setting generally is about 5 mg/kg weekly parenterally when administered with probenecid and special efforts to ensure adequate hydration, or about 2 mg/kg weekly parenterally without probenecid and special efforts to ensure adequate hydration. The maximum non-nephrotoxic doses of other HSNAs are determined by routine preclinical or clinical experiments well within the ordinary skill in the art as described above. Thus, in most circumstances, a cHPMPC dosage

of greater than about 3 mg/kg/week or biweek administered parenterally to humans will be antivirally effective and non-nephrotoxic. At this dose, it is not believed to be necessary to use probenecid or to hydrate the patients. However, dosages in excess of about 4, 5, 6, 10, 15, 20, 25, 30, 35, 40, 45 or 50 mg/kg/week or biweek also may be suitable under the circumstances. These doses, particularly above 10 mg, optionally will be accompanied by hydration (drinking fluids or administration of intravenous fluids). Probenecid also may be of value at these doses. The greatest non-nephrotoxic dose of cHPMPC that can be used in humans is believed to be on the order of 50 mg/kg/week, but will vary based on the same parameters as the minimum dose, and may extend to 100 mg/kg/week. For the most part these dosages are given as a single weekly dose, i.e. one administration of cHPMPC/week. In humans, as little as 1 mg/kg/week or biweek of cHPMPC is used without probenecid or, optionally hydration.

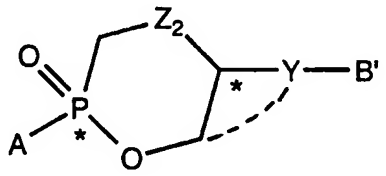
In determining the proper dose, one also needs to take into account whether the cHSNA is an intermediate that is converted *in vivo* into the free acid, i.e., whether the phosphonate hydroxyl group(s) are unsubstituted or are substituted by esters or amides. In general, the dosage of an intermediate form of cHSNA will be higher than that of the free hydroxyl HSNA, taking into account the bioavailability of the intermediate by oral intake and its greater molecular weight. The dosage of this invention for a given cHSNA intermediate is determined readily by assaying the proportion of free cHSNA generated in the plasma upon administration of the intermediate, generally by the oral route. The intermediate will be administered so as to emulate the desired cHSNA plasma concentration previously obtained by intravenous or other systemic administration routes. Analogous reasoning is applied to topical routes of administration, where the benchmark is the tissue concentration at the topical site of delivery. In an illustrative example of the foregoing principles, if the maximum non-nephrotoxic dose of the uncyclized HSNA by intravenous administration is 1 mg/kg/day, then the intravenous dose of the cHSNA will be greater than 1 mg/kg/day (and ordinarily greater than 2 mg/kg/day). If the intermediate form of the cHSNA is 50% bioavailable upon oral administration and is 3 times the molecular weight of the corresponding HSNA, then the oral dose of the cHSNA intermediate will be greater than about 6 mg/kg/day. The

determination of bioavailability for such compounds is conventional and well within the ordinary skill in the art.

Alternatively, it will be within the skill of the ordinary artisan to determine the MND for other HPMPB or HSNA compounds than cHPMPC by simply elevating the dosages until evidence of nephrotoxicity (2+ proteinuria or elevated creatinine) or other dose-limiting cytotoxicity is detected as described above. In general, initial dosages will be in the range of about 0.5 mg/kg to 10 mg/kg administered 1, 2 or 7 times a week, and thereafter the amounts are increased until toxicity is evident. Usually, only 1 or 2 animal species are studied, e.g., rats or guinea pigs, to arrive at non-cytotoxic candidate doses for humans in accord with conventional practice.

HSNA Intermediates

Intermediates for the cyclized HSNA compounds are useful in the practice of the therapeutic method of this invention. Such intermediates have structure (VIIIa):

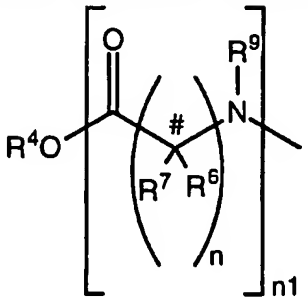


(VIIIa)

wherein Z_2, A, Y, *, and B' are defined above.

Suitable A substituents are amidates or esters which may, but need not be, hydrolyzable *in vivo*. Those which are not hydrolyzable *in vivo* are useful as intermediates for *in vitro* hydrolytic conversion to the free acids. While the cHSNA compounds (Ia), (IIa), (IIIa), (IVa), (Va), (VIa) and (VIIa) optionally are administered orally, typically they will be administered orally in the form of compounds of structure (VIIIa). Those that are hydrolyzable *in vivo* are useful as prodrugs. If not, they are intermediates for cHSNAs for use in the therapeutic method of this invention.

A is OD^1, SD^1, NHR^{40} (where R^{40} is defined above), or



(IX)

n is an integer having a value from 1 to 5 and if n > 1, each $-C(R^3)(R^2)-$ may be the same or different;

n1 is an integer;

substituents linked to the carbon atom designated # are in the *R*, *S* or *RS* configuration;

D^1 is H, (a) C_1-C_{20} alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N and halogen (F, Cl, Br, I), (b) C_3-C_{20} aryl (usually C_3-C_6) which is unsubstituted or substituted by substituents independently selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl (1 to 3 halogen atoms), cyano, nitro, OH, O, N and halogen, (c) C_4-C_{20} aryl-alkyl which is unsubstituted or substituted in the aryl moiety by substituents independently selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl (1 to 3 halogen atoms), cyano, nitro, OH, O, N and halogen, (d) C_3-C_{24} 1-acyloxy-1-alkyl (C_1-C_8 alkyl), (e) C_6-C_{24} 1-acyloxy-1-aryl-1-alkyl (C_1-C_6 aryl, C_1-C_4 alkyl), (f) C_3-C_{24} 1-acyloxy-2-alkoxy-1-alkyl (C_1-C_8 alkyl), (g) C_3-C_{24} 1-acyloxy-2-haloalkyl (C_1-C_8 haloalkyl, 1 to 3 halogen atoms), (h) a saccharide residue, (i) a glyceride lipid residue, (j) C_2-C_{20} alkenyl or alkynyl, (k) C_2-C_{10} alkyoxyalkyl, (l) C_4-C_{10} (usually C_3-C_6) heteroaryl, (m) C_5-C_{20} alkaryl, (n) C_5- C_{20} alkoxyalkaryl, (o) C_5-C_{20} alkheteroaryl, (p) $-CH_2C(O)NR_4$, (q) $-CH_2C(O)OR_4$, (r) $-CH_2OC(O)R_4$, (s) $-CH(R_4)OC(O)R_4$, (t) $-C(R_4)HC(O)N(R_4)_2$, (u) $-C(R_4)HC(O)NH(R_4)$, (v) $-CH_2C(R_4)_2CH_2OH$, or (w) C_5-C_{20} alkoxyalkheteroaryl groups, or the same groups in which at least one (ordinarily 1-3) hydrogen atom is substituted with amino, hydroxyl, carboxyl, $-OR_4$, $-COOR_4$, $-CON(R_4)_2$, $-CONH(R_4)$, $-CONH_2$, $-NO_2$, $-CX_3$, $-OCX_3$, -CN, $-N_3$, or halo, where X is halo or hydrogen but at least one X is halo;

R^4 is H or is C_3-C_9 alkyl which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen, C_3-C_6 aryl which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen or C_3-C_9 aryl-alkyl which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen;

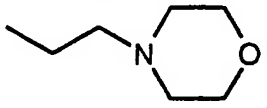
R^6 is H or C_1-C_9 alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^4$ and halogen, C_3-C_6 aryl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^4$ and halogen or C_3-C_9 aryl-alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^4$ and halogen;

R^7 is C(O)-OR^4, amino, amide, guanidinyl, imidazolyl, indolyl, sulfoxide, phosphoryl, C_1-C_3 alkylamino, C_1-C_3 alkyldiamino, C_1-C_6 alkenylamino, hydroxy, thiol, C_1-C_3 alkoxy, C_1-C_3 alkthiol, $(CH_2)_nCOOR^4$, C_1-C_6 alkyl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_{10} alkoxyphenyl; C_2-C_6 alkenyl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_{10} alkoxyphenyl; C_6-C_{12} aryl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_{10} alkoxyphenyl;

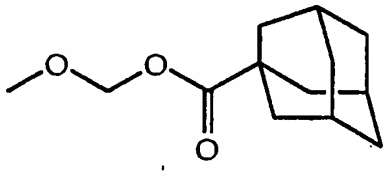
R^9 is H, D^2, -O-N-methylpiperidinyl, C_1-C_9 alkyl which is unsubstituted (e.g. methyl, ethyl or propyl) or substituted by OH, O, N, $COOR^4$ or halogen, C_3-C_6 aryl which is unsubstituted or substituted by OH, O, N, $COOR^4$ or halogen, or C_3-C_9 aryl-alkyl which is unsubstituted or substituted by OH, O, N, $COOR^4$ or halogen; R^9 usually is H, but may be taken together with R^7 to form prolyl.

D^1 also includes phenyl, 2- and 3-pyrrolyl, 2- and 3-thienyl, 2- and 4-imidazolyl, 2-, 4- and 5-oxazolyl, 3- and 4-isoxazolyl, 2-, 4- and 5-thiazolyl, 3-, 4- and 5-isothiazolyl, 3- and 4-pyrazolyl, 2-, 3- and 4-pyridinyl, 2-, 4- and 5-pyrimidinyl, 2-, 3- and 4-alkoxyphenyl (C_1-C_{12} alkyl including 2-, 3- and 4-methoxyphenyl and 2-, 3- and 4-ethoxyphenyl), 2-, 3- and 4-halophenyl (including 2-, 3- and 4-fluorophenyl), 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dihalophenyl (including 2,4-difluorophenyl and 2,4-dichlorophenyl),

2-, 3- and 4-haloalkylphenyl (1 to 5 halogen atoms, C_1-C_{12} alkyl including 2-, 3- and 4-trifluoromethylphenyl and 2-, 3- and 4-trichloromethylphenyl), 2-, 3- and 4-cyanophenyl, carboalkoxyphenyl (C_1-C_4 alkyl including 2-, 3- and 4-carboethoxyphenyl (-C_6H_4-C(O)-OC_2H_5) and 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dicarboethoxyphenyl), 1-, 2-, 3-, and 4-pyridinyl (-C_5H_4N), 2-, 3- and 4-nitrophenyl, 2-, 3- and 4-haloalkylbenzyl (1 to 5 halogen atoms, C_1-C_{12} alkyl including 4-trifluoromethylbenzyl), alkylsalicylphenyl (C_1-C_4 alkyl including 2-, 3- and 4-ethylsalicylphenyl), 2-,3- and 4-acetylphenyl, 1,8-dihydroxy-naphthyl (-O-$C_{10}H_6$-OH), 2,2'-dihydroxybiphenyl (-O-C_6H_4-C_6H_4-OH); alkoxy ethyl [C_1-C_6 alkyl including -CH_2-CH_2-O-CH_3 (methoxy ethyl) and phenoxymethyl], aryloxy ethyl [C_6-C_9 aryl (including phenoxy ethyl) or C_6-C_9 aryl substituted by OH, NH_2, halo, C_1-C_4 alkyl or C_1-C_4 alkyl substituted by OH or by 1 to 3 halo atoms], -C_6H_4-CH_2-N(CH_3)2, N-ethylmorpholino

( ; -$(CH_2)_2$-N[$(CH_2)_2(CH_2)_2$]O),

adamantoyl oxymethyl, pivaloyloxy(methoxyethyl)methyl (-CH($CH_2CH_2OCH_3$)-O-C(O)-C(CH_3)3),

( ; -O-CH_2-O-C(O)-$C_{10}H_{15}$),

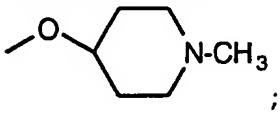
pivaloyloxymethyl (-CH_2-O-C(O)-C(CH_3)3), pivaloyloxy(methoxymethyl)-methyl (-CH(CH_2OCH_3)-O-C(O)-C(CH_3)3), pivaloyloxyisobutyl (-CH(CH(CH_3)2)-O-C(O)-C(CH_3)3) isobutyryloxymethyl (-CH_2-O-C(O)-CH_2-CH(CH_3)2), cyclohexanoyl oxymethyl (-CH_2-O-C(O)-C_6H_{11}), benzyl (-CH_2-C_6H_5), isopropyl (-CH(CH_3)2), t-butyl (-C(CH_3)3), -CH_2-CH_3, -$(CH_2)_2$-CH_3, -$(CH_2)_3$-CH_3, -$(CH_2)_4$-CH_3, -$(CH_2)_5$-CH_3, -CH_2-CH_2F, -CH_2-CH_2Cl, -CH_2-CF_3, -CH_2-CCl_3, NHR^{10}, $N(R^{10})_2$, or R^5;

wherein R^5 is $CH_2C(O)N(R^{10})_2$, $CH_2C(O)OR^{10}$, $CH_2OC(O)R^{10}$, $CH(R^{10})OC(O)R^{10}$, $CH_2C(R^{10})_2CH_2OH$, CH_2OR^{10}, NH-CH_2-C(O)O-CH_2CH_3, N(CH_3)-CH_2-C(O)O-CH_2CH_3, NHR^{40}, CH_2-O-C(O)-C_6H_5, CH_2-O-C(O)-$C_{10}H_{15}$, -CH_2-O-C(O)-CH_2CH_3 , CH_2-O-C(O)-CH(CH_3)2, CH_2-O-C(O)-C(CH_3)3, CH_2-O-C(O)-CH_2-C_6H_5;

and wherein R^{10} is C_1-C_{20} alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N and halogen (1 to 5 halogen atoms), C_6-C_{20} aryl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N and halogen (1 to 5 halogen atoms) or C_7-C_{20} aryl-alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N and halogen (1 to 5 halogen atoms);

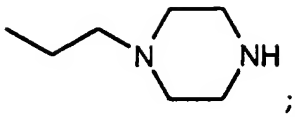
provided that for compounds of formulas $N(R^{10})_2$, $CH_2C(O)N(R^{10})_2$, $CH_2C(O)OR^{10}$, $CH_2OC(O)R^{10}$, $CH(R^{10})OC(O)R^{10}$ and $CH_2C(R^{10})_2CH_2OH$, the total number of carbon atoms present is less than 25 (preferably the number of carbon atoms present is about 4 to about 14) and R^{40} is C_1-C_{20} alkyl. In general, A is not SD^1 or NHR^{40}, or OD^1 where D^1 is alkyl.

The invention compounds containing structure (IX) are optionally alkylated at the α-nitrogen atom of the amino acid by the R^9 group defined above. Exemplary R^9 groups include H, CH_3, CH_2CH_3, benzyl, 4-O-N-methylpiperidinyl



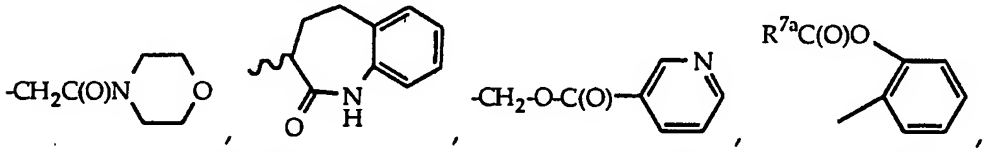
(; -O-CH[$(CH_2)_2(CH_2)_2$]N(CH_3)), and 3-O-N-methylpiperidinyl.

The intermediates containing structure (IX) are optionally esterified at the amino acid carboxyl moiety by the R^4 group defined above. Exemplary R^4 groups include H, methyl, ethyl, propyl, isopropyl, butyl, t-butyl ($C(CH_3)_3$), phenyl (-C_6H_5), benzyl (-CH_2-C_6H_5), 1-pyridyl, 3-pyridyl, 1-pyrimidinyl, N-ethylmorpholino (-CH_2-CH_2-N[$(CH_2)_2(CH_2)_2$]O), N-2-propylmorpholino (-CH(CH_3)-CH_2-N[$(CH_2)_2(CH_2)_2$]O), methoxyethyl (-CH_2-CH_2-O-CH_3), 4-N-methylpiperidyl (-CH[$(CH_2)_2(CH_2)_2$]N(CH_3)), 3-N-methylpiperidyl, phenol which is 2-, 3-, or 4- substituted by $N(R^{30})_2$ where R^{30} is independently H or C_1-C_6 alkyl unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^4$ and halogen or C_6-C_{12} aryl unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^4$, $N(R^{7a})_2$ (wherein R^{7a} is H or C_1-C_4 alkyl) and halogen (including 2-, 3-, and 4-N,N-dimethylaminophenol and 2-, 3-, and 4-N,N-diethylaminophenol), 1-ethylpiperazinyl



[; -CH_2-CH_2-NC_4H_8NH], and N^4-substituted 1-ethyl-piperazinyl (-$(CH_2)_2$-N[$(CH_2)_2(CH_2)_2$]NR^6, where R^6 is as defined above).

D^1 also is 2,3-dihydro-6-hydroxyindene, sesamol, catechol monoester, -CH_2-C(O)-N$(R^{7a})_2$ wherein each R^{7a} is the same or different, -CH_2-S(O)(R^{7a}), -CH_2-$S(O)_2$(R^{7a}), -O-CH_2-CH(OC(O)CH_2R^{7a})-CH_2(OC(O)CH_2R^{7a}), cholesteryl, a 5 or 6 carbon monosaccharide, such as ∝-D-galactose, ∝-D-glucose or ∝-D-fructose; enolpyruvate (HOOC-C(=CH_2)O-); glycerol; or D-∝,β-diglyceride (the fatty acids composing glyceride lipids generally are naturally occurring saturated or unsaturated C_6-C_{26} fatty acids such as linoleic, lauric, myristic, palmitic, stearic, oleic, palmitoleic, linolenic and the like fatty acids); trimethoxybenzyl, triethoxybenzyl, 2-alkyl pyridinyl (C_{1-4} alkyl),



C_3-C_6 aryl (including phenyl, 2- and 3-pyrrolyl, 2- and 3-thienyl, 2- and 4-imidazolyl, 2-, 4- and 5-oxazolyl, 3- and 4-isoxazolyl, 2-, 4- and 5-thiazolyl, 3-, 4- and 5-isothiazolyl, 3- and 4-pyrazolyl, 2-, 3- and 4-pyridinyl and 2-, 4- and 5-pyrimidinyl) substituted by 3, 4 or 5 halogen atoms or 1 or 2 atoms or groups selected from halogen, C_1-C_{12} alkoxy (including methoxy, ethoxy, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethoxy and 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-diethoxy substituted phenyl), cyano, nitro, OH, C_1-C_{12} haloalkyl (1 to 6 halogen atoms), C_1-C_{12} alkyl (including methyl and ethyl), C_2-C_{12} alkenyl or C_2-C_{12} alkynyl; C_1-C_4 alkylene-C_3-C_6 aryl (including benzyl, -CH_2-pyrrolyl, -CH_2-thienyl, -CH_2-imidazolyl, -CH_2-oxazolyl, -CH_2-isoxazolyl, -CH_2-thiazolyl, -CH_2-isothiazolyl, -CH_2-pyrazolyl, -CH_2-pyridinyl and -CH_2-pyrimidinyl) substituted in the aryl moiety by 3 to 5 halogen atoms or 1 to 2 atoms or groups selected from halogen, C_1-C_{12} alkoxy (including methoxy and ethoxy), cyano, nitro, OH, C_1-C_{12} haloalkyl (1 to 6 halogen atoms; including -CH_2-CCl_3), C_1-C_{12} alkyl (including methyl and ethyl), C_2-C_{12} alkenyl or C_2-C_{12} alkynyl.

As used herein, and unless modified by the immediate context: 1) the term alkyl, alkenyl and alkynyl refer to straight chain, branched and cyclic residues. Thus, C_1-C_4 alkyl includes methyl, ethyl, propyl,

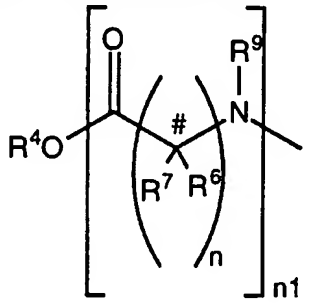
cyclopropyl, isopropyl, n-, sec-, iso- and tert-butyl, cyclobutyl and the like while alkenyl includes ethenyl, propenyl, isopropenyl, 1-, 2- and 3-butenyl, 1- and 2-isobutenyl and the like. The term alkyl also includes cyclic N-, S- or O- heterocarbonyl (such as piperidyl and morpholino). 2) The term aryl includes N-, S- or O- heteroaryl, including phenyl, 2- and 3-pyrrolyl, 2- and 3-thienyl, 2- and 4-imidazolyl, 2-, 4- and 5-oxazolyl, 3- and 4-isoxazolyl, 2-, 4- and 5-thiazolyl, 3-, 4- and 5-isothiazolyl, 3- and 4-pyrazolyl, 2-, 3- and 4-pyridinyl, 2-, 4- and 5-pyrimidinyl. When "O" or "N" are substituted into aryl or alkyl this means that a ring or chain methyne or methylene is replaced by O, N or NH. Acyloxy means alkyl- or aryl-C(O)O-.

Group A includes an amino acid residue or peptide. Amino acid residues or peptides are linked to the phosphorus atom through an epsilon or alpha amino group, thereby producing a phosphoramidate bond. The amino acid residue is any moiety comprising at least one carboxyl and at least one amino residue directly linked by at least one intervening carbon atom, typically a single (α) carbon atom, while peptides are polymers of two or more of such amino acids. Any amino acid is suitably employed as an A group provided that it is capable of autocatalytically hydrolyzing the amidate bond. Thus, it must contain, or must, upon being converted (hydrolyzed) *in vivo* contain, a free carboxyl group. In general, the amino acids corresponding to the residues employed in the compounds of this invention are naturally occurring and have no pharmacological activity. However, optimal pharmacokinetic activity (substantially complete autocatalytic hydrolysis upon hydrolysis of the distal amide or ester bond) may be achieved by the use of non-naturally occurring amino acid residues.

A variety of intervening structures located between the carboxyl and amino (amidate) groups of the amino acids are suitable. All that is necessary is that the intervening group have sufficient conformation and length as to not prevent the acid catalysis of the phosphoroamidate bond and release of the phosphonate by the carboxyl group (which in turn is produced *in vivo*, e.g. by deesterification, deamidation or peptidolytic cleavage of a carboxyl ester or amide of the amino acid residue). In general, the intervening structure may be as simple as methylene (when the residue is glycyl) or substituted methylene (other α amino acids). The structure ordinarily contains up to about 5 carbon or heteroatoms in the direct linkage between the carboxyl carbon and the amidate nitrogen, as for

example in the case of intervening ethylene, propylene, butylene, or pentylene groups or their substituted analogs, such as for example oxyesters in which O replaces carbon and, as appropriate, hydrogen. An example of such an intervening structure would be -CH-O-CH(R^6)(R^7)-, where R^6 and R^7 are defined above. In general, fewer intervening atoms are employed when more rapid hydrolysis is desired, although it will be understood that larger structures are suitable if they possess sufficient flexibility or have conformers in which the carboxyl group is positioned in proximity to the amidate bond.

In general, the amino acid residues of structure (IX) for use herein have the structure shown in structure (IX).



(IX)

Ordinarily, n is 1 or 2, R^6 is H and R^7 is 1-guanidinoprop-3-yl, benzyl, 4-hydroxybenzyl, imidazol-4-yl, indol-3-yl, methoxyphenyl, ethoxyphenyl, or the side group or atom of a naturally occurring amino acid such as H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2-CH(CH_3)_2$, $-CHCH_3-CH_2-CH_3$, $-CH_2-C_6H_5$, $-CH_2CH_2-S-CH_3$, $-CH_2OH$, $-CH(OH)-CH_3$, $-CH_2-SH$, $-CH_2-C_6H_4OH$, -CH2-CO-NH_2, $-CH_2-CH_2-CO-NH_2$, $-CH_2-COOH$, $-CH_2-CH_2-COOH$, $-(CH_2)_4-NH_2$, $-(CH_2)_3-[R^9]$, and $-(CH_2)_3-NH-C(NH_2)-NH_2$. With respect to the carboxyl-containing side chains of naturally occurring amino acids such as glutamic and aspartic acid, it will be understood that if the C atom of the amino acid carboxyl group is linked by 5 or less atoms to the phosphoramide N atom then the carboxyl optionally will be blocked, e.g. by esterification or amidation.

When the amino acid residues contain one or more chiral centers, any of the D or L isomers, or mixtures thereof are suitable. In general, if the HSNA intermediate is to be hydrolyzed non-enzymatically *in vivo*, D isomers should be used. On the other hand, L isomers may be more versatile since they can be susceptible to both non-enzymatic as well as potential targeted enzymatic hydrolysis, and may be more efficiently

transported by amino acid or dipeptidyl transport systems in the gastrointestinal tract.

Examples of suitable amino acid residues include the following:

Glycyl;

Aminopolycarboxylic acids, e.g., aspartic acid, β-hydroxyaspartic acid, glutamic acid, β-hydroxyglutamic acid, β-methylaspartic acid, β-methylglutamic acid, β,β-dimethylaspartic acid, γ-hydroxyglutamic acid, β,γ-dihydroxyglutamic acid, β-phenylglutamic acid, γ-methyleneglutamic acid, 3-aminoadipic acid, 2-aminopimelic acid, 2-aminosuberic acid and 2-aminosebacic acid residues;

Amino acid amides such as glutaminyl and asparaginyl;

Polyamino- or polybasic-monocarboxylic acids such as arginine, lysine, β-aminoalanine, γ-aminobutyrine, ornithine, citruline, homoarginine, homocitrulline, 5-hydroxy-2,6-diaminohexanoic acid (commonly, hydroxylysine, including allohydroxylysine) and diaminobutyric acid residues;

Other basic amino acid residues such as histidinyl;

Diaminodicarboxylic acids such as α,α'-diaminosuccinic acid, α,α'-diaminoglutaric acid, α,α'-diaminoadipic acid, α,α'-diaminopimelic acid, α,α'-diamino-β-hydroxypimelic acid, α,α'-diaminosuberic acid, α,α'-diaminoazelaic acid, and α,α'-diaminosebacic acid residues;

Imino acids such as proline, 4- or 3-hydroxy-2-pyrrolidinecarboxylic acid (commonly, hydroxyproline, including allohydroxyproline), γ-methylproline, pipecolic acid, 5-hydroxypipecolic acid, $-N([CH_2]_nCOOR^4)_2$, wherein n and R^4 are as defined above, and azetidine-2-carboxylic acid residues;

A mono- or di-alkyl (typically C_1-C_8 branched or normal) amino acid such as alanine, valine, leucine, allylglycine, butyrine, norvaline, norleucine, heptyline, α-methylserine, α-amino-α-methyl-γ-hydroxyvaleric acid, α-amino-α-methyl-δ-hydroxyvaleric acid, α-amino-α-methyl-ε-hydroxycaproic acid, isovaline, α-methylglutamic acid, α-aminoisobutyric acid, α-aminodiethylacetic acid, α-aminodiisopropylacetic acid, α-aminodi-n-propylacetic acid, α-aminodiisobutylacetic acid, α-aminodi-n-butylacetic acid, α-aminoethylisopropylacetic acid, α-amino-n-propylacetic acid, α-aminodiisoamyacetic acid, α-methylaspartic acid, α-methylglutamic acid, 1-aminocyclopropane-1-carboxylic acid; isoleucine,

alloisoleucine, tert-leucine, β-methyltryptophan and α-amino-β-ethyl-β-phenylpropionic acid residues; β-phenylserinyl;

Aliphatic α-amino-β-hydroxy acids such as serine, β-hydroxyleucine, β-hydroxynorleucine, β-hydroxynorvaline, and α-amino-β-hydroxystearic acid residues;

α-Amino, α-, γ-, δ- or ε-hydroxy acids such as homoserine, γ-hydroxynorvaline, δ-hydroxynorvaline and epsilon-hydroxynorleucine residues; canavinyl and canalinyl; γ-hydroxyornithinyl;

2-hexosaminic acids such as D-glucosaminic acid or D-galactosaminic acid residues;

α-Amino-β-thiols such as penicillamine, β–thiolnorvaline or β-thiolbutyrine residues;

Other sulfur containing amino acid residues including cysteine; homocystine; β-phenylmethionine; methionine; S-allyl-L-cysteine sulfoxide; 2-thiolhistidine; cystathionine; and thiol ethers of cysteine or homocysteine;

Phenylalanine, tryptophan and ring-substituted α amino acids such as the phenyl- or cyclohexylamino acids α-aminophenylacetic acid, α-aminocyclohexylacetic acid and α-amino-β-cyclohexylpropionic acid; phenylalanine analogues and derivatives comprising aryl, lower alkyl, hydroxy, guanidino, oxyalkylether, nitro, sulfur or halo-substituted phenyl (e.g., tyrosine, methyltyrosine and o-chloro-, p-chloro-, 3,4-dicloro, o-, m- or p-methyl-, 2,4,6-trimethyl-, 2-ethoxy-5-nitro, 2-hydroxy-5-nitro and p-nitro-phenylalanine); furyl-, thienyl-, pyridyl-, pyrimidinyl-, purine or naphthylalanines; and tryptophan analogues and derivatives including kynurenine, 3-hydroxykynurenine, 2-hydroxytryptophan and 4-carboxytryptophan residues;

α-Amino substituted amino acid residues including sarcosine (N-methylglycine), N-benzylglycine, N-methylalanine, N-benzylalanine, N-methylphenylalanine, N-benzylphenylalanine, N-methylvaline and N-benzylvaline; and

α-Hydroxy and substituted α-hydroxy amino acid residues including serine, threonine, allothreonine, phosphoserine and phosphothreonine residues.

Of particular interest are hydrophobic residues such as mono-or di-alkyl or aryl amino acids, cycloalkylamino acids (proline) and the like.

These hydrophobic residues, together with R^4, contribute to cell permeability by increasing the partition coefficient of the cHSNA. Typically, the residue will not contain a sulfhydryl or guanidino substituent.

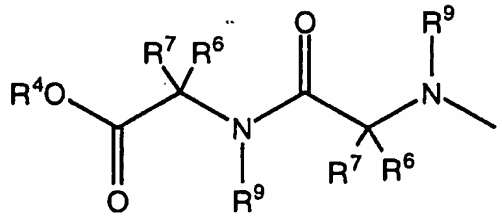
If n1 is greater than 1, then Group A is a polypeptide radical, including dipeptides, or polypeptides of 3, 5, 10 or 100 or more residues. For the most part, dipeptides not containing aspartic or glutamic acid in the residue adjacent to the P atom will not autocatalytically hydrolyze the amidate bond and therefore the carboxyl groups (generally 1 or 2) in the distal residue do not need to be esterified or amidated, i.e., R^4 can be H in these circumstances. However, if such compounds are intended to be used as precursors for the free phosphonate nucleotide analog *in vivo*, rather than as immunogens for example, the polypeptides ordinarily will contain a peptidolytic enzyme cleavage site at the peptide bond linking the first residue and the next residue distal to the phosphorus atom. Such cleavage sites are flanked by enzymatic recognition structures, e.g. particular residues recognized by a peptidolytic enzyme.

Peptidolytic enzymes are well known, and in particular include carboxypeptidases. Carboxypeptidases digest polypeptides by removing C-terminal residues, and are specific in many instances for particular C-terminal sequences. Such enzymes and their substrate requirements in general are well known. For example, a dipeptide having a given pair of residues and a free carboxyl terminus is covalently bonded through its ∝-amino group to the phosphorus atom of the HSNA's of this invention. It is expected that this peptide will be cleaved by the appropriate dipeptidase or protease, leaving the carboxyl of the proximal amino acid residue to autocatalytically cleave the amidate bond.

Examples of dipeptidyl groups (designated by their single letter code) include AA, AR, AN, AD, AC, AE, AQ, AG, AH, AI, AL, AK, AM, AF, AP, AS, AT, AW, AY, AV, RA, RR, RN, RD, RC, RE, RQ, RG, RH, RI, RL, RK, RM, RF, RP, RS, RT, RW, RY, RV, NA, NR, NN, ND, NC, NE, NQ, NG, NH, NI, NL, NK, NM, NF, NP, NS, NT, NW, NY, NV, DA, DR, DN, DD, DC, DE, DQ, DG, DH, DI, DL, DK, DM, DF, DP, DS, DT, DW, DY, DV, CA, CR, CN, CD, CC, CE, CQ, CG, CH, CI, CL, CK, CM, CF, CP, CS, CT, CW, CY, CV, EA, ER, EN, ED, EC, EE, EQ, EG, EH, EI, EL, EK, EM, EF, EP, ES, ET, EW, EY, EV, QA, QR, QN, QD, QC, QE, QQ, QG, QH, QI, QL, QK, QM, QF, QP, QS, QT, QW, QY, QV, GA, GR, GN, GD, GC, GE, GQ, GG, GH, GI, GL, GK,

GM, GF, GP, GS, GT, GW, GY, GV, HA, HR, HN, HD, HC, HE, HQ, HG, HH, HI, HL, HK, HM, HF, HP, HS, HT, HW, HY, HV, IA, IR, IN, ID, IC, IE, IQ, IG, IH, II, IL, IK, IM, IF, IP, IS, IT, IW, IY, IV, LA, LR, LN, LD, LC, LE, LQ, LG, LH, LI, LL, LK, LM, LF, LP, LS, LT, LW, LY, LV, KA, KR, KN, KD, KC, KE, KQ, KG, KH, KI, KL, KK, KM, KF, KP, KS, KT, KW, KY, KV, MA, MR, MN, MD, MC, ME, MQ, MG, MH, MI, ML, MK, MM, MF, MP, MS, MT, MW, MY, MV, FA, FR, FN, FD, FC, FE, FQ, FG, FH, FI, FL, FK, FM, FF, FP, FS, FT, FW, FY, FV, PA, PR, PN, PD, PC, PE, PQ, PG, PH, PI, PL, PK, PM, PF, PP, PS, PT, PW, PY, PV, SA, SR, SN, SD, SC, SE, SQ, SG, SH, SI, SL, SK, SM, SF, SP, SS, ST, SW, SY, SV, TA, TR, TN, TD, TC, TE, TQ, TG, TH, TI, TL, TK, TM, TF, TP, TS, TT, TW, TY, TV, WA, WR, WN, WD, WC, WE, WQ, WG, WH, WI, WL, WK, WM, WF, WP, WS, WT, WW, WY, WV, YA, YR, YN, YD, YC, YE, YQ, YG, YH, YI, YL, YK, YM, YF, YP, YS, YT, YW, YY, YV, VA, VR, VN, VD, VC, VE, VQ, VG, VH, VI, VL, VK, VM, VF, VP, VS, VT, VW, VY and VV, wherein the amidate bond is formed with the second residue.

Exemplary dipeptidyl A groups have the structure of formula (X) wherein R^6 is H, R^7 independently are the side chains of a naturally occurring amino acid, and R^4 and R^9 independently are as defined above.



(X)

Tripeptides also are useful. The A group sequence -X^1ProX^2 (where X^1 is any amino acid residue and X^2 is an amino acid residue, a carboxyl ester of Pro or H) will be cleaved by luminal carboxypeptidase to yield X^1 with a free carboxyl, which in turn autocatalytically cleaves the phosphono amidate bond. X^2 usually will be a benzyl ester of the carboxy group of X^2. Thus, n1 usually is 1, 2 or 3, but may range up to 5, 10 or 100 or more residues.

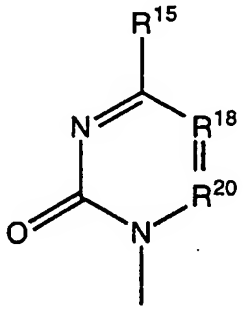
If the amino acid residue has 2 or more amine groups, e.g., in the case of lysinyl, arginyl or ornithinyl residues, then R^7 represents the group -$[C(R^{11})_2]_{n2}N(R^6)$- where n2 is 0 to 6, R^{11} is H, C_1-C_{20} alkyl, C_6-C_{20} aryl, C_7-

C_{20} alkylaryl, C_7-C_{20} arylalkyl, C_1-C_{20} alkoxy, C_6-C_{20} aryloxy or hydroxyl, R^6 is defined above, and the N atom of R^7 is bonded to the phosphorus atom of a structure (VIIIa) compound. Such compounds thus will contain a plurality of phosphonate moieties, i.e., group A is polysubstituted with cHSNA. For example when both the epsilon (ε)/delta (δ) and alpha (α) amino groups of lysine or ornithine are substituted with HSNA moieties the amidate contains and is believed to be capable of releasing two molecules of active drug, each expected to emerge under different pharmacokinetics and therefore further sustaining the drug release.

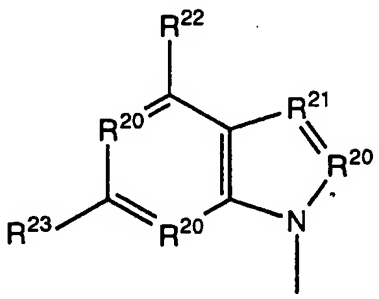
Heterocyclic Bases

The heterocyclic base generally is an unsubstituted nitrogen-, or nitrogen and sulfur- containing heterocyclic ring structure or such ring structure substituted with from 1 to 3 substituents independently selected from oxo, hydroxy, amino, protected amino, fluoro, chloro, bromo, iodo, C_1-C_9 haloalkyl (1-3 halo), C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 haloalkenyl, C_1-C_9 alkoxy, C_2-C_9 thioalkenyl, C_1-C_9 alkylthiol, amino C_1-C_9 alkyl, amino C_3-C_4 alkenyl, amino C_3-C_4 alkynyl, cycloamino C_2-C_5 alkyl, thio C_1-C_9 alkyl, C_1-C_9 hydroxyalkyl, C_1-C_3 alkoxy, C_1-C_4 alkyl, acylamine, thiol, =S, or $=N\text{-}NH_2$.

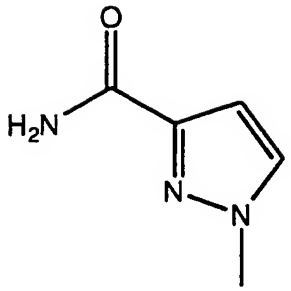
Typically, B' is selected from structures (XI)-(XIV) and (Xa.1) - (XIIIa.1).



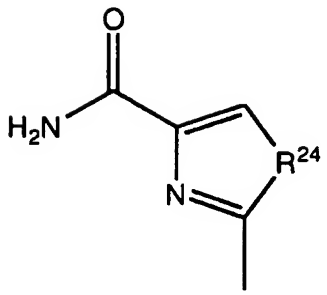
(XI)



(XII)



(XIII)



(XIV)

wherein R^{15} is H, OH, F, Cl, Br, I, OR^{16}, SH, SR^{16}, NH_2, or NHR^{17};

R^{16} is C_1-C_6 alkyl or C_2-C_6 alkenyl including CH_3, CH_2CH_3, CH_2CCH (2-propynyl), CH_2CHCH_2 (2-allyl), C_3H_7;

R^{17} is C_1-C_6 alkyl or C_2-C_6 alkenyl including CH_3, CH_2CH_3, CH_2CCH, CH_2CHCH_2, C_3H_7;

R^{18} is N, CF, CCl, CBr, CI, CR^{19} or CSR^{19}, COR^{19};

R^{19} is H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2 - C_9 alkynyl, C_1-C_9 alkyl-C_1-C_9 alkoxy, or C_7-C_9 aryl-alkyl unsubstituted or substituted by OH, F, Cl, Br or I including CH_3, CH_2CH_3, -$CHCH_2$, -CHCHBr, CH_2CH_2Cl, CH_2CH_2F, -CH_2CCH, -CH_2CHCH_2, C_3H_7, CH_2OH, CH_2OCH_3, $CH_2OC_2H_5$, -CH_2OCCH, -$CH_2OCH_2CHCH_2$, $CH_2C_3H_7$, CH_2CH_2OH, $CH_2CH_2OCH_3$, $CH_2CH_2OC_2H_5$, -CH_2CH_2OCCH, -$CH_2CH_2OCH_2CHCH_2$, $CH_2CH_2OC_3H_7$;

R^{20} is N or CH;

R^{21} is N, CH, CCN, CCF_3, CC≡CH or $CC(O)NH_2$;

R^{22} is H, OH, NH_2, SH, SCH_3, SCH_2CH_3, SCH_2CCH, SCH_2CHCH_2, SC_3H_7, $NH(CH_3)$, $N(CH_3)_2$, $NH(CH_2CH_3)$, $N(CH_2CH_3)_2$, $NH(CH_2CCH)$, $NH(CH_2CHCH_2)$, $NH(C_3H_7)$ or halogen (F, Cl, Br or I);

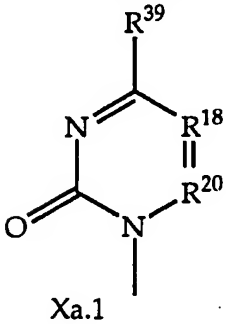
R^{23} is H, OH, F, Cl, Br, I, SCH_3, SCH_2CH_3, SCH_2CCH, SCH_2CHCH_2, SC_3H_7, OR^{16}, NH_2, or NHR^{17}; and

R^{24} is O, S or Se.

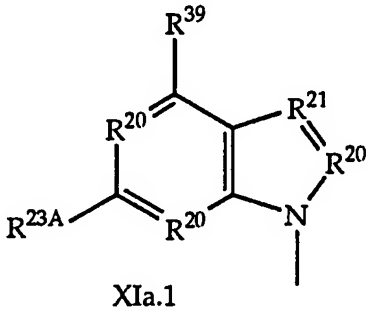
B' includes both protected and unprotected bases certain of which are described above. Protecting groups for exocyclic amines and other labile groups are known (Greene et al. "Protective Groups in Organic Synthesis") and include N-benzoyl, isobutyryl, 4,4'-dimethoxytrityl (DMT) and the like. The selection of protecting group will be apparent to the ordinary artisan and will depend upon the nature of the labile group and

the chemistry which the protecting group is expected to encounter, e.g. acidic, basic, oxidative, reductive or other conditions. Exemplary protected species are N^4-benzoylcytosine, N^6-benzoyladenine, N^2-isobutyrylguanine and the like.

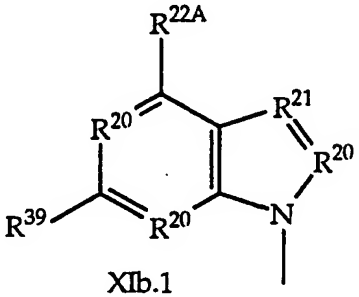
Protected bases have the formulas Xa.1, XIa.1, XIb.1, XIIa.1 or XIIIa.1



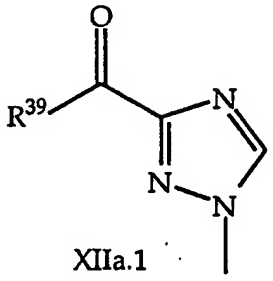
Xa.1



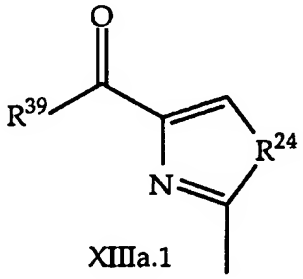
XIa.1



XIb.1



XIIa.1



XIIIa.1

wherein R^{18}, R^{20}, R^{21}, R^{24} have the meanings previously defined; R^{22A} is R^{39} or R^{22} provided that R^{22} is not NH_2; R^{23A} is R^{39} or R^{23} provided that R^{23} is not NH_2; R^{39} is NHR^{40}, $NHC(O)R^{36}$ or $CR^{41}N(R^{38})_2$ wherein R^{36} is C_1-C_{19} alkyl, C_1-C_{19} alkenyl, C_3-C_{10} aryl, adamantoyl, alkylaryl, or C_3-C_{10} aryl substituted with 1 or 2 atoms or groups selected from halogen, methyl, ethyl, methoxy, ethoxy, hydroxy and cyano; R^{38} is C_1-C_{10} alkyl, or both R^{38} together are 1-morpholino, 1-piperidine or 1-pyrrolidine; and R^{41} is hydrogen or CH_3. For bases of structures XIa.1 and XIb.1, if R^{39} is present at R^{22A} or R^{23A}, both R^{39} groups on the same base will generally be the same. Exemplary R^{36} are phenyl, phenyl substituted with one of the foregoing R^{36} aryl substituents, $-C_{10}H_{15}$ (where $C_{10}H_{15}$ is 2-adamantoyl), $-CH_2-C_6H_5$, $-C_6H_5$, $-C(CH_3)_3$, $-CH(CH_3)_2$, $-CH_2CH_3$, methyl, ethyl, butyl, t-butyl, heptanyl, nonanyl, undecanyl, undecenyl and the like.

Exemplary R^{40} include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, hexyl, octyl and decanyl).

In general, B' for compounds of structure (VIIa), (IIa), (IIIa), (VIa), (Ia), (Va) and (IVa) will be structure (XI) or (XII). Specific bases include hypoxanthine, inosine, thymine, uracil, xanthine, 8-aza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 7-deaza-8-aza derivatives of adenine, guanine, 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 1-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 7-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 3-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 6-azacytosine; 5-fluorocytosine; 5-chlorocytosine; 5-iodocytosine; 5-bromocytosine; 5-methylcytosine; 5-bromovinyluracil; 5-fluorouracil; 5-chlorouracil; 5-iodouracil; 5-bromouracil; 5-trifluoromethyluracil; 5-methoxymethyluracil; 5-ethynyluracil; 5-propynyluracil and the like.

Preferably, B' is a 9-purinyl residue selected from guanyl, 3-deazaguanyl, 1-deazaguanyl, 8-azaguanyl, 7-deazaguanyl, adenyl, 3-deazaadenyl, 1-dezazadenyl, 8-azaadenyl, 7-deazaadenyl, 2,6-diaminopurinyl, 2-aminopurinyl, 6-chloro-2-aminopurinyl and 6-thio-2-aminopurinyl, or a B' is a 1-pyrimidinyl residue selected from cytosinyl, 5-halocytosinyl, and 5-(C_1-C_3-alkyl)cytosinyl.

Methods of Manufacture of HSNAs

cHPMPC and the cyclic analogues of other HSNAs are prepared by a number of methods from the free hydroxy phosphonic acid. These methods include treatment with DCC in DMF (Ho et al., op cit.), reaction with Vilsmeier's reagent ($ClCH=N(CH_3)_2Cl$), or methods of phosphate activation known per se. The cHSNAs are prepared by direct dehydration of the corresponding HPMP nucleotide analog using DCC (dicyclohexylcarbo-diimide) or using 4-morpholino-N,N'-dicyclohexylcarboxamide as described by (Ho et al. "Mol Pharmacol." 41:197-202 [1992]). The cyclic phosphonate is condensed with an optionally protected amino acid ester in the presence of a 1:1 mixture of

triphenylphosphine and 2,2'-dipyridyl disulfide in a suitable solvent such as pyridine or DMF. In another embodiment of this invention for the preparation of cHSNA from the corresponding HSNA, the HSNA is (a) treated with $ClCH=N(CH_3)_2Cl$ to yield the phosphonylchloridate and (b) optionally the phosphonylchloridate is reacted with a nucleophile (preferably at low temperature, e.g. lower than about -20°C) such as an alcohol or amine to produce one of the intermediates described above. In a further step the product of steps (a) or (b) are subject to hydrolysis or protonolysis (typically acid protonolysis) respectively to yield the cHSNA (treatment of the product of step (a)) or its intermediate (treatment of the product of step (b)). Vilsmeier's reagent is advantageously produced *in situ* by combining $SOCl_2$, PCl_5, $POCl_3$, $COCl_2$ or the like with DMF. Advantageously, the product of step (a) is not purified or separated from the reaction mixture before being reacted with the nucleophile, a distinct economic advantage for this synthetic route. The compounds of structure (Ia) and (Va) are readily made from their uncyclized counterparts by the same methods, e.g. treatment with DCC in DMF.

Substituted and unsubstituted alkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl and other D^1 esters and amidates of cHSNA typically are made by reacting the appropriate HSNA with $SOCl_2$/DMF to yield the activated phosphonylchloride (see Scheme 1), followed by treatment with the corresponding nucleophile (e.g. alkoxide, phenolate, amine, etc.) to yield the protected intermediate formamidine which is subsequently hydrolyzed to the target compound. Alternatively, esters can also be prepared as depicted in Scheme 2. The N-,O- protected intermediate phosphonate diester is obtained from the three building blocks by known methods. The N- and O- protecting groups are subsequently removed followed by treatment of the phosphonate diester 3 with NaH leading to cyclization yielding target compound 4. A third method for the synthesis of cHSNA esters entails alkylation of the cHSNA using common alkylating agents D^1L (where L is a leaving group) such as alkyl halides, tosylates, diazoalkanes and the like (see Scheme 3). This method is particularly useful for preparing acyloxyalkyl esters by treatment of the cHSNA with the corresponding acyloxyalkylhalide. In an exemplary method for the preparation of acyloxyalkyl esters of cHSNAs, shown in more detail in Example 8, DCC and $R^4C(O)OCH_2Cl$ are reacted with the cHSNA; but in contradistinction with prior methods the stoichiometric

proportion of DCC: $R^4C(O)OCH_2Cl$, cHSNA is 1-2:1-2:1. Use of such low proportions of reactants lessens side reactions with any exocyclic amino group of B' and thereby greatly improves yields.

An alternative reaction suitable for synthesizing most amidate compounds is converting a nucleotide analog phosphonate to the corresponding chloridate by reaction with thionyl chloride in solvent (DMF) as described in EP 481 214. An amino acid, dipeptide or other molecule bearing a free amine is then reacted with the chloridate to yield the corresponding bis-amidate.

Synthesis of cHSNA amino acid amidate compounds containing amino, guanidino or carboxyl groups (such as lys, arg, his, asn, gln, lys-lys, arg-arg, lys-arg and the like) is accomplished by the same method, but using protected amine or carboxyl groups. After synthesis of the protected bis-amidate compound, the protecting groups are removed by conventional methods. Suitable protecting groups are well known and include acid labile groups such as p-tosyl, BOC (t-butoxycarbonyl) and FMOC (fluorene methoxycarbonyl) for protecting amine groups. Groups such as t-butyl, methyl, ethyl, benzyl and the like can be used to protect carboxyl groups. These groups can be removed under acid, base or hydrogenolysis conditions or can be removed with an esterase according to conventional methods.

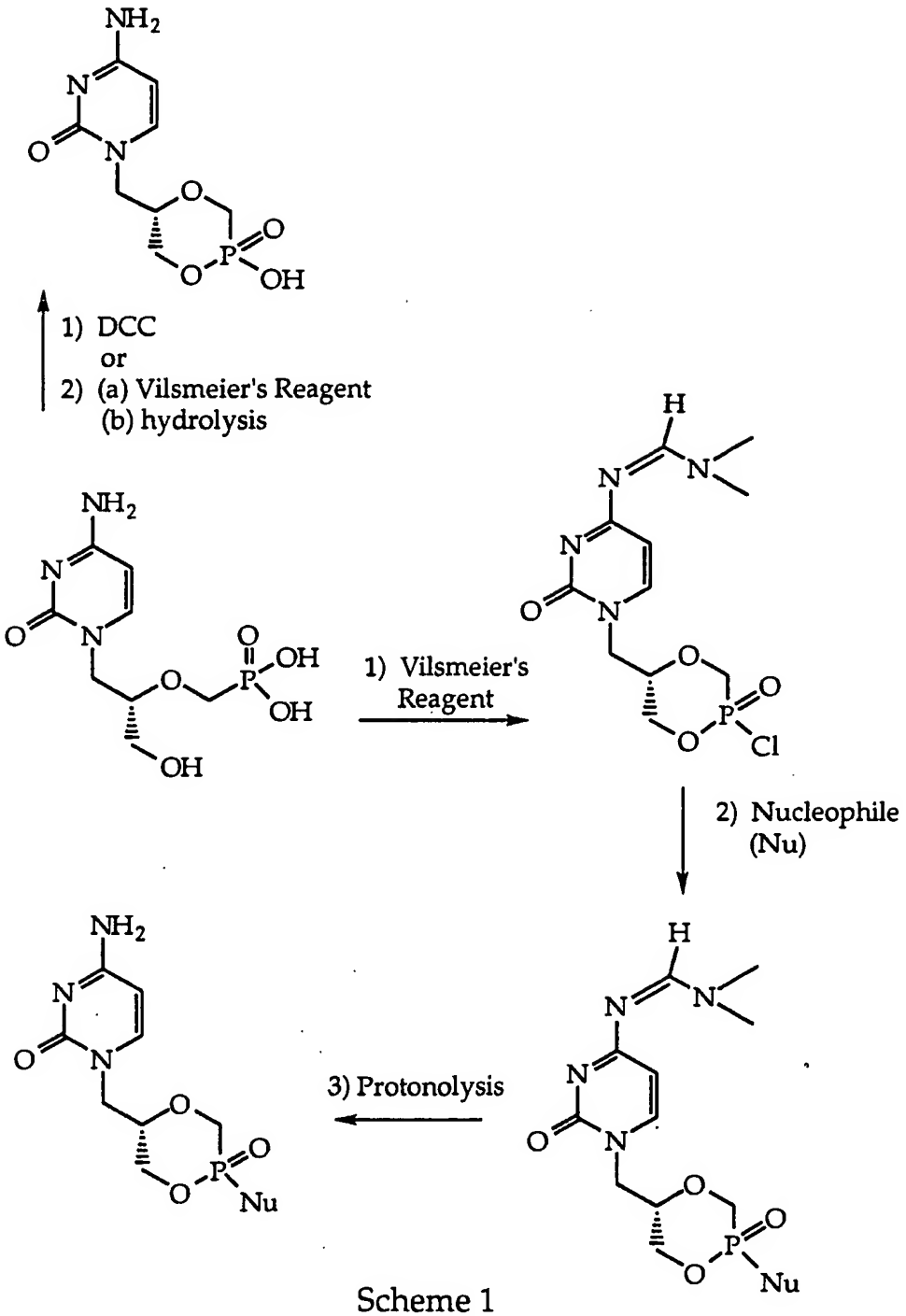
Synthesis of cHSNA amino acid amidate compounds containing amino acids such as tyr, cys, ser and thr is accomplished by optionally protecting hydroxyl or thiol groups using protecting groups know in the art. For example, the hydroxyl group of ser, thr or tyr can be protected using benzyl, ethyl and the like and the thiol group of cys can be protected using trityl, p-methylbenzyl and the like. The choice of a protecting group will depend on the stability of the bis-amidate toward conditions used to remove a particular protecting group. Appropriate protecting groups can be selected or determined by the skilled artisan using routine methods.

Dipeptide or tripeptide species can be selected on the basis of known transport properties and/or susceptibility to peptidases that can affect transport to intestinal mucosal or other cell types. Dipeptides and tripeptides lacking an α-amino group are transport substrates for the peptide transporter found in brush border membrane of intestinal mucosal cells (Bai, J.P.F., "Pharm Res", 9:969-978 [1992]). Transport competent peptides can thus be used to enhance bioavailability of bis

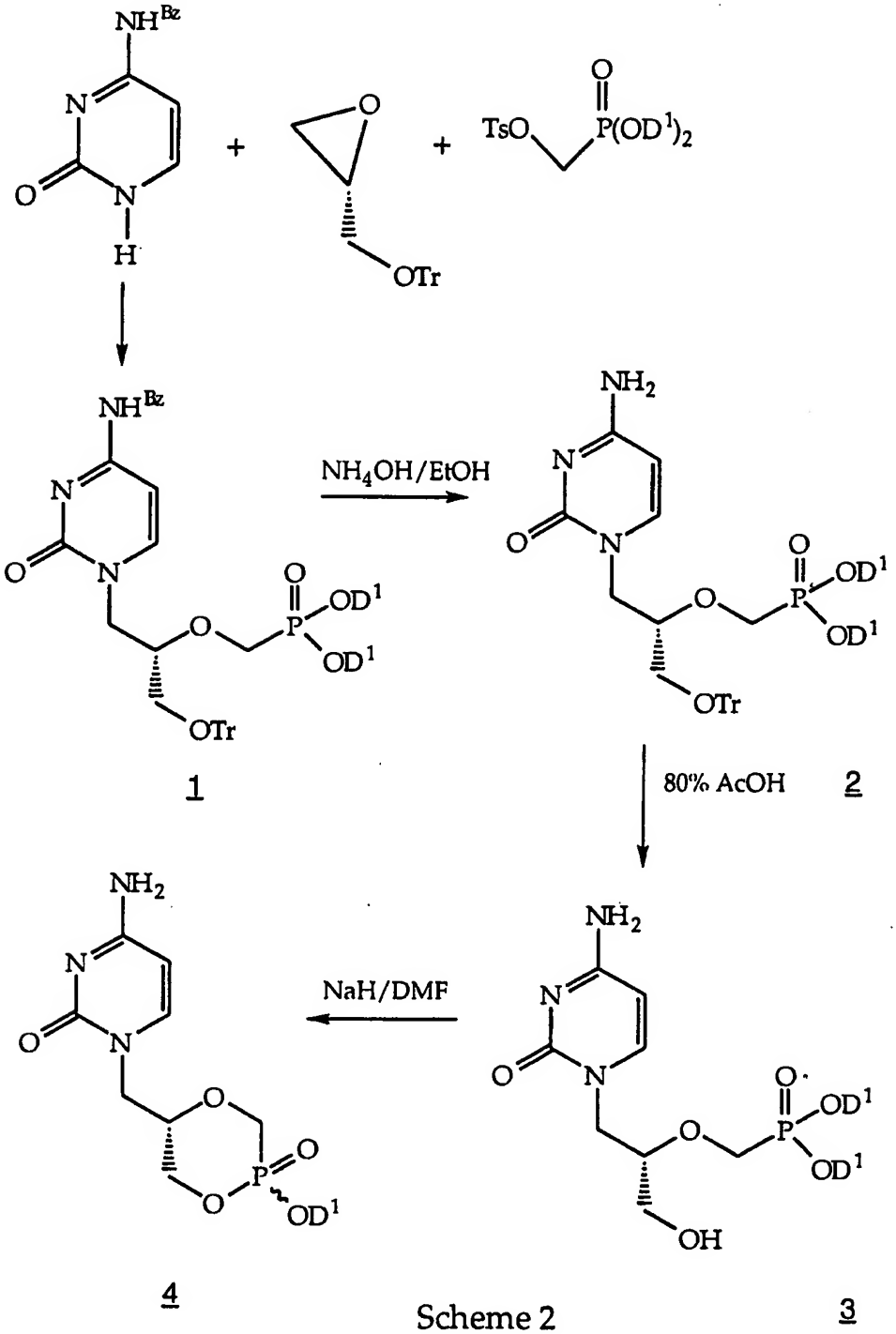
amidate compounds. Di- or tripeptides having one or more amino acids in the D configuration are also compatible with peptide transport and can be utilized in amidate compounds. Amino acids in the D configuration can be used to reduce the susceptibility of a di- or tripeptide to hydrolysis by proteases common to the brush border such as aminopeptidase N (EC 3.4.11.2). In addition, di- or tripeptides with amino acid residues can be selected on the basis of their relative resistance to hydrolysis by proteases found in the lumen of the intestine. For example, tripeptides or oligopeptides lacking asp and/or glu are poor substrates for aminopeptidase A (EC 3.4.11.7) and di- or tripeptides lacking amino acid residues on the N-terminal side of hydrophobic amino acids (leu, tyr, phe, val, trp) are poor substrates for endopeptidase 24.11 (EC 3.4.24.11) while peptides lacking a pro residue at the penultimate position at a free carboxyl terminus are poor substrates for carboxypeptidase P (EC .3.4.17). Similar considerations can also be applied to the selection of peptides that are either relatively resistant or relatively susceptible to hydrolysis by cytosolic, renal, hepatic, serum or other peptidases.

Synthesis of N-alkylamine amidates (where $-NHR^{40}$ is linked to the phosphorus atom and R^{40} is C_{1-20} alkyl, including C_{4-16} alkyl) is accomplished essentially as described (Saito, "Chem. Pharm. Bull.", 39:3207 [1991]).

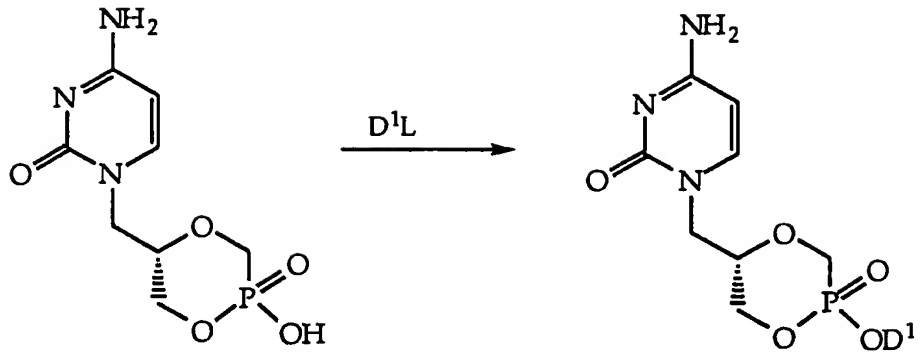
Each of the following schemes exemplify HPMPC as the HSNA. However, any B' is employed in place of cytosine, provided that any exocyclic oxo or amino groups are protected as required. Also, step 3 of scheme 1 obviously will be omitted when B' contains no exocyclic amine. In addition, any HSNA Y group is employed in place of the $-CH_2-$ of HPMPC, or both the Y group and B' are substituted to produce esters or amides of the HSNAs described herein.


1) DCC
or
2) (a) Vilsmeier's Reagent
(b) hydrolysis
1) Vilsmeier's Reagent
2) Nucleophile (Nu)
3) Protonolysis

Scheme 1

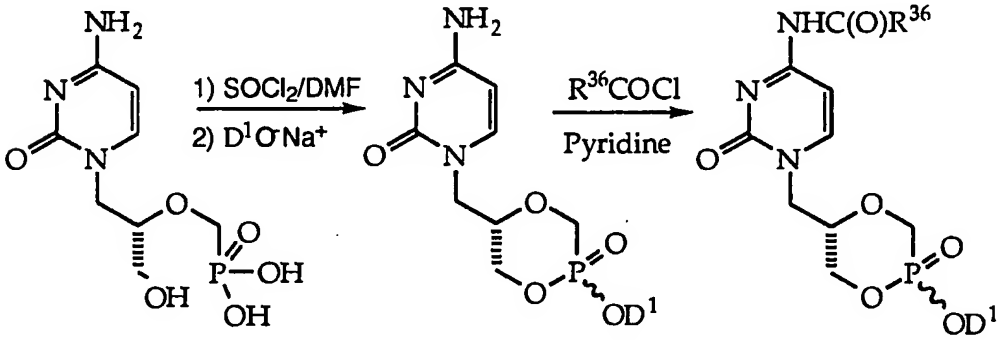

NH^Bz
N
O
N
H
+
O
OTr
+
TsO
P(OD^1)_2
NH^Bz
N
O
N
O
P
OD^1
OD^1
OTr
NH_4OH/EtOH
NH_2
N
O
N
O
P
OD^1
OD^1
OTr
1
80% AcOH
2
NH_2
N
O
N
O
P
OD^1
OD^1
OH
NaH/DMF
NH_2
N
O
N
O
O
P
O
OD^1
4
3

Scheme 2

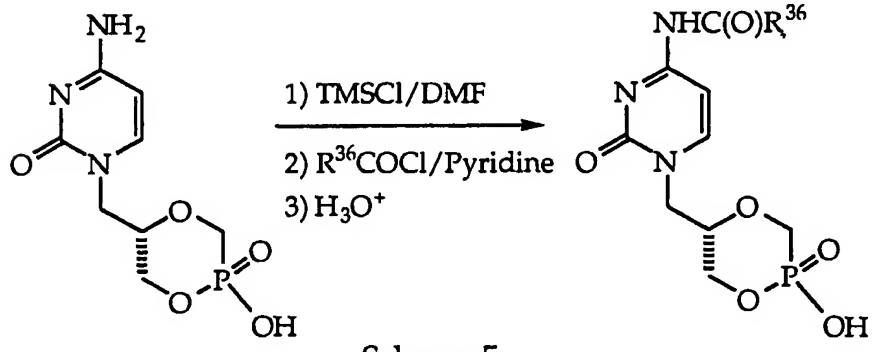


Scheme 3

Two other methods for making the intermediates herein use the following procedures (Schemes 4 and 5, illustrated for the cytosine or protected cytosine base)



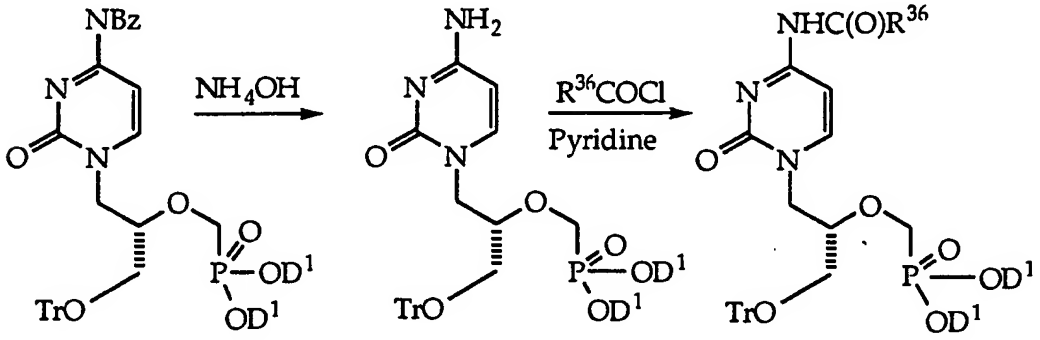
Scheme 4

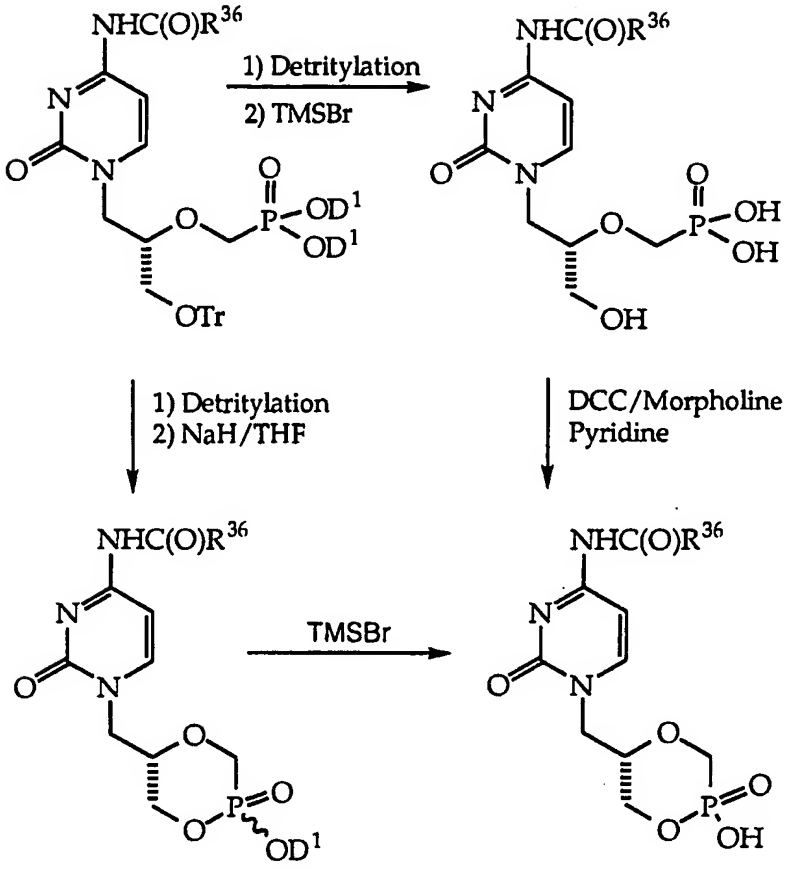


Scheme 5

wherein D^1 and R^{36} are defined above. Either procedure is readily adapted to synthesizing compounds containing amino bases other than cytosine, e.g., adenine, guanine, 2,6-diaminopurine or 2-aminopurine. The first step of Scheme 4 is readily adapted to compounds containing bases other than cytosine. The second step is useful in preparing base protected analogues of compounds containing bases with exocyclic amino, e.g. cytosine, adenine, 2,6-diaminopurine or 2-aminopurine, described above. The amide linkage is conveniently formed by reaction of the acyl chloride with the exocyclic amine of the base. When D^1 is linked to the free phosphonate the resulting ester will comprise a single isomer or a racemic mixture at the phosphorus atom. Low temperature reaction conditions (lower than about -20°, e.g., -20° to -40°C) generally results in a scalemic mix while reaction at higher temperatures (above about -20°, e.g. -20° to 40°C) generally results in a racemic mix. When a scalemic mixture is obtained, the isomers can be conveniently separated by HPLC, although the mixture can be used for example as a synthetic intermediate or as an active antimicrobial agent, without resolution. See Example 7.

Another method to obtain bases comprising the $C(O)R^{36}$ protecting group is accomplished as follows using the acyl chloride ($R^{36}C(O)Cl$) using HPMPC and cHPMPC as an example

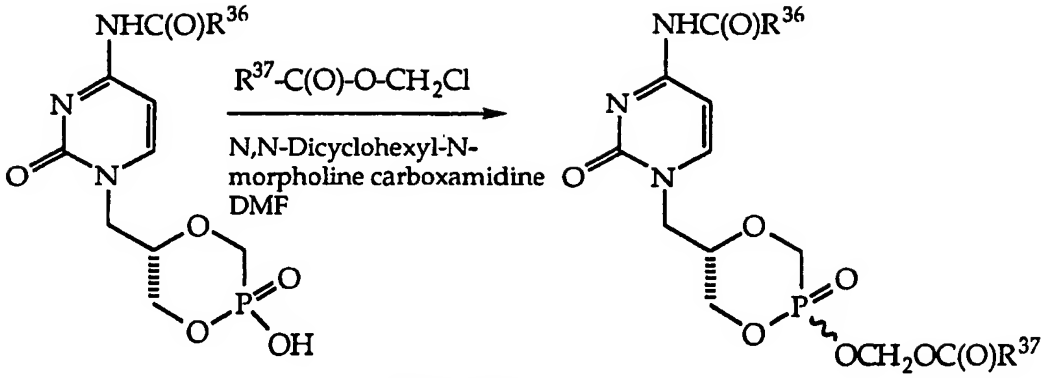




Scheme 6

wherein Tr is the hydroxyl protecting group trityl. The detritylation step is accomplished by acid treatment, such as 80% acetic acid at about 10° to 60°C for 1-2 hours. The D^1 moiety is removed using a Lewis acid such as TMSBr to yield the free phosphonate.

cHSNAs having protected amine bases and acyloxymethyl esters of the phosphonate moiety are produced as follows:

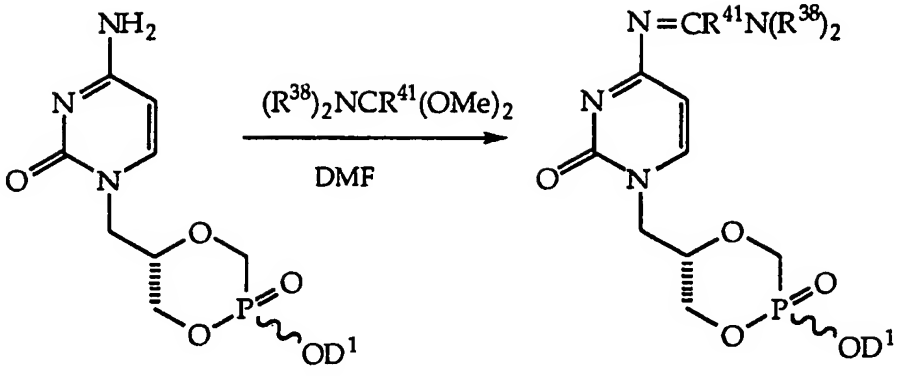


Scheme 7

wherein R^{37} is C_1-C_{20} alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl (1 to 3 halogen atoms), cyano, nitro, OH, O, NH and halogen (including ethyl, propyl, isopropyl, t-butyl, isobutyl and adamantoyl), or C_3-C_{10} aryl which is unsubstituted or substituted by substituents independently selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl (1 to 3 halogen atoms), cyano, nitro, OH, O, N and halogen (including phenyl, and 3- or 4-pyridyl).

The cHSNA ester synthesis of Scheme 7 as applied to the pivaloyloxymethyl ester of cHPMPC yields a scalemic mixture at the phosphorus atom. The mixture was separated by HPLC into the two isomers which were then exposed to an rat intestinal homogenate or to a rat intestinal wash. One of the isomers was converted to cHPMPC after incubation in the homogenate while the other isomer was converted to HPMPC pivaloyloxymethyl monoester. Both isomers were converted to HPMPC pivaloyloxymethyl monoester after incubation in the intestinal wash. These results suggested that (1) in at least some cases, enzyme activity can have a differential effect on the metabolic fate of a cHPMPC ester depending on which phosphorus isomer is present and (2) chemical activity (i.e., the acidity of the intestinal wash) can affect the metabolic fate of a given compound in a manner that differs from enzyme activity.

The amine protecting group $=CR^{41}N(R^{38})_2$ is incorporated into an exocyclic amine to yield protected base compounds as follows



Scheme 8

Exemplary R^{38} alkyl groups include methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl and cyclobutyl. In general, R^{38} both alkyl groups will be the same. The reaction can be carried out in dry DMF at room temperature (about 20-30°C) as previously described (Kerr et al., "J. Pharm. Sci.", 83:582 [1994]; Kerr et al., "J. Med. Chem.", 35:1996 [1992]), or DMF can be substituted with CH_3CN and 4 Å molecular sieves. Exemplary compounds include species where D^1 is hydrogen, alkyl (including ethyl, propyl, isopropyl), aryl (including phenyl) or acyloxymethyl. Protected bases where R^{41} is hydrogen are stable under neutral anhydrous conditions and are generally labile under acidic aqueous conditions. When R^{41} is methyl, the protecting group is more stable to aqueous acidic or basic conditions.

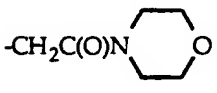
Compounds containing a protected base and 1 or 2 amino acids, dipeptides or oligopeptides attached to the phosphorus atom via an amidate linkage are obtained as described for synthesis of amidate-ester compounds.

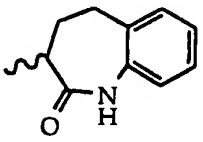
Table 2A lists D^1 ester and A amidate moieties that can be incorporated into the phosphorus atom of cHSNAs. Esters of structures 1-5, 8-10 and 16, 17, 19-22 are synthesized by reacting a nucleotide analog (such as cHPMPC) the corresponding halide (chloride or acyl chloride and the like) and N ,N-dicylohexyl-N-morpholine carboxamidine (or another base such as DBU, triethylamine, $CsCO_3$, N,N-dimethylaniline and the like) in DMF (or other solvent such as acetonitrile or N-methylpyrrolidone). Esters of structures 5-7, 11, 12, 21, and 23-26 are synthesized by reaction of the alcohol or alkoxide salt (or the corresponding amines in the case of compounds such as 13, 14 and 15)

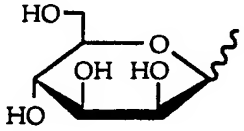
with a nucleotide analog monochlorophosphonate or dichlorophosphonate (such as cHPMPC monochlorophosphonate or another activated phosphonate).

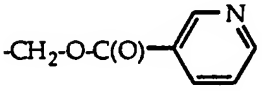
TABLE 2A

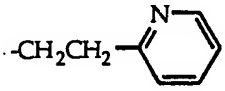
1. $-CH_2-C(O)-N(R^{7b})_2$
2. $-CH_2-S(O)(R^{7b})$
3. $-CH_2-S(O)_2(R^{7b})$
4. $-CH_2-O-C(O)-CH_2-C_6H_5$
5. 3-cholesteryl
6. 3-pyridyl
7. N-ethylmorpholino
8. $-CH_2-O-C(O)-C_6H_5$
9. $-CH_2-O-C(O)-CH_2CH_3$
10. $-CH_2-O-C(O)-C(CH_3)_3$
11. $-CH_2-CCl_3$
12. $-C_6H_5$
13. $-NH-CH_2-C(O)O-CH_2CH_3$
14. $-N(CH_3)-CH_2-C(O)O-CH_2CH_3$
15. $-NHR^{40}$
16. $-CH_2-O-C(O)-C_{10}H_{15}$
17. $-CH_2-O-C(O)-CH(CH_3)_2$
18. $-CH_2-C\#H(OC(O)CH_2R^{7b})-CH_2-(OC(O)CH_2R^{7b})$

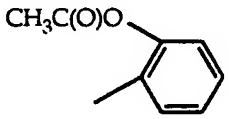
19. 

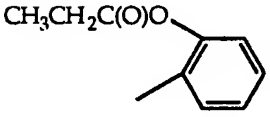
20. 

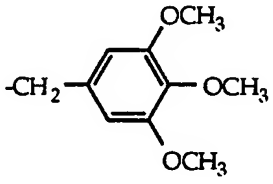
21. 

22. 

23. 

24. 

25. 

26. 

R^{7b} is the same or different and is H or C_1 - C_4 alkyl (including methyl, ethyl, propyl, isopropyl and t-butyl).

The final products of Schemes 4-8 (and their analogues with other amino bases) and the compounds of Table 2A are useful for preparing cHSNAs or they can be orally or parenterally administered as such.

Salts

The compounds of this invention optionally are supplied as salts. Those salts which are pharmaceutically acceptable are of particular interest since they are useful in administering the foregoing compounds for medical purposes. Salts which are not pharmaceutically acceptable are useful in manufacturing processes, for isolation and purification purposes, and in some instances, for use in separating stereoisomeric forms of the compounds of this invention. The latter is particularly true of amine salts prepared from optically active amines.

Pharmaceutically acceptable metal and amine salts are useful herein and include salts which are stable under ambient conditions and which contain nontoxic cations. Suitable metal salts include the sodium, potassium, calcium, barium, zinc, and aluminum salts. The sodium and potassium salts are preferred. Suitable amine salts are prepared from amines which have sufficient basicity to form a stable salt, and preferably include those amines which are frequently used in medicinal chemistry because of their low toxicity and acceptability for medical use. These include ammonium and the trialkylamines such as triethylamine, and others including procaine, dibenzylamine, N-benzyl-beta-phenethylamine, ephenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N-ethylpiperidine, benzylamine, basic amino acids, e.g. lysine and arginine, and dicyclohexylamine.

Acid addition salts are formed with the compounds of the invention in which a basic function such as an amino, alkylamino, or dialkylamino group is present as a substituent on B'. The pharmaceutically acceptable, i.e., nontoxic, acid addition salts are preferred. They are chosen optimally to be compatible with the customary pharmaceutical vehicles and adapted for oral or parenteral administration. Some suitable acids for use in the preparation of such salts are hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, various organic carboxylic and sulfonic acids, such as acetic acid, citric acid, propionic acid, succinic acid, benzoic acid, tartaric acid, fumaric acid, mandelic acid, ascorbic acid, malic acid, methanesulfonic acid, toluenesulfonic acid, fatty acids, and others.

Pharmaceutical Formulations

Compounds of the invention and their pharmaceutically, i.e. physiologically, acceptable salts (hereafter collectively referred to as the active ingredients), as well as known compounds for use in the therapeutic method herein, are administered by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including ocular, buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). It will be appreciated that the preferred route may vary with for example the condition of the recipient.

While it is possible for the active ingredients to be administered alone it is preferably to present them as pharmaceutical formulations. The formulations, both for veterinary and for human use, of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the patient.

The formulations include those suitable for topical or systemic administration, including oral, rectal, nasal, buccal, sublingual, vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations are in unit dosage form and are prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

For infections of the eye or other external tissues, e.g. mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the emulsifying wax,

and the wax together with the oil and fat make up the emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween® 60, Span® 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is suitably present in such formulations in a concentration of 0.01 to 20%, in some embodiments 0.1 to 10%, and in others about 1.0% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for nasal or inhalational administration wherein the carrier is a solid include a powder having a particle size for

example in the range 1 to 500 microns (including particle sizes in a range between 20 and 500 microns in increments of 5 microns such as 30 microns, 35 microns, etc). Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient. Formulations suitable for aerosol administration may be prepared according to conventional methods and may be delivered with other therapeutic agents. Inhalational therapy is readily administered by metered dose inhalers.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration are sterile and include aqueous and non-aqueous injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials with elastomeric stoppers, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as recited above, or an appropriate fraction thereof, of an active ingredient.

In addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The present invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefor.

Veterinary carriers are materials useful for the purpose of administering the composition to cats, dogs, horses, rabbits and other

animals and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered orally, parenterally or by any other desired route.

Compounds of the invention can be used to provide controlled release pharmaceutical formulations containing a matrix or absorbent material and as active ingredient one or more compounds of the invention in which the release of the active ingredient can be controlled and regulated to allow less frequent dosing or to improve the pharmacokinetic or toxicity profile of the compound. Controlled release formulations adapted for oral administration in which discrete units comprising one or more compounds of the invention can be prepared according to conventional methods.

Uses for Novel Compounds

Any of the compounds and therapeutic method described herein are used in the treatment or prophylaxis of various microbial infections in man or animals, particularly bacterial, parasitic, protozoan or viral infections caused by microbial species including DNA viruses, RNA viruses, *Plasmodium*, *Pneumocystis*, herpesviruses (CMV, HSV 1, HSV 2, VZV, and the like), retroviruses, hepadnaviruses, (e.g. HBV), papillomavirus, hantavirus, adenoviruses and HIV or HIV-related opportunistic infections and related conditions such as tuberculosis, malaria, pneumocystis pneumonia, and CMV retinitis. Other infections to be treated with the compounds and methods herein include MSV, RSV, SIV, FIV, MuLV, and other retroviral infections of rodents and other animals.

Novel compounds described herein are useful as intermediates in the preparation of detectable labels for oligonucleotide probes. The compounds are hydrolyzed to yield the diacid, diphosphorylated and then incorporated into an oligonucleotide by conventional enzymatic or chemical means. The incorporated base from the compound of the invention will be capable of participating in base pairing and thus will not interfere substantially with the binding of the oligonucleotide to its complementary sequence (E. De Clercq "Rev. Med. Virol." 3:85-96 [1993]); should it interfere with oligonucleotide binding to complementary sequence, the compound of the invention is incorporated as the 3'

terminal base, an innocuous position and a conventional site for oligonucleotide labeling. The sugar analogue donated by the compound of this invention that is incorporated into the oligonucleotide is detected by any means, such as NMR or immune recognition.

Example 1

5 Day Repeat Dose Toxicity Study of HPMPC and cHPMPC Administered Intravenously to Rats

Groups of Sprague-Dawley rats (5 to 7 weeks of age; five rats per sex per group; Charles River Laboratories, Wilmington, Mass.) were administered solutions of drugs or saline by tail vein injection at 10 ml/kg of body weight once daily for 5 days. Solutions were HPMPC (10 mg/ml) or cHPMPC (10 and 25 mg/ml). Doses were 100 mg of HPMPC/kg or 100 or 250 mg of cHPMPC/ug as a once daily intravenous injection. Saline was the control solution. Animals were observed daily for clinical signs during the dosing phase and for an additional 10 days post-dosing. At 10 days postdosing, blood was withdrawn for analysis of hematology, enzyme, and chemical parameters. Animals were then sacrificed and necropsied, and the kidneys were removed and fixed for histopathologic evaluation.

No animals died during the 5 days of dosing or the 10 days of follow-up. However, group body weights, body weight gains, and food consumption were decreased relative to those of the controls in both the 100 mg/kg HPMPC and 250 mg/kg cHPMPC groups, with the severity being less in the latter group.

In the group given 100 mg of HPMPC per kg, histopathologic evaluation showed that the kidneys in both males and females had degenerative changes characterized by tubular depletion and degeneration, tubular cytomegaly, tubular karyomegaly, and tubular degeneration. In the more severely affected kidneys, there was a loss of renal tubules in the outer cortical region. In the groups given cHPMPC at either 100 or 250 mg/kg, no treatment-related changes in the kidneys were seen.

Example 2

14-Day Intravenous Toxicity Study of cHPMPC in Rats

Groups of rats treated with cHPMPC at 10, 40, or 150 mg/kg given intravenously daily for 14 days were compared with a control group given saline. In this study groups of Sprague-Dawley rats (five per sex per group) were administered drug or saline by tail vein injection at 10 ml/kg once daily for 14 days. Solutions of cHPMPC were prepared at 1, 4 and 15 mg/ml. Animals were observed daily for clinical signs. At day 15, blood was withdrawn for analysis of hematology, enzyme, and chemical parameters. Animals were then sacrificed and necropsied, and tissues from 19 different organs was removed and preserved for histopathologic evaluation. The kidney changes in rats given 150 mg of cHPMPC per kg/day were characterized by tubular depletion and degeneration accompanied by tubular cytomegaly and tubular karyomegaly and an increased incidence of severe tubular dilation and regeneration. Loss of tubules was also seen in the more severely affected kidneys. At 40 mg of cHPMPC per kg/day, treatment-related changes were limited to a minimal tubular cytomegaly and tubular karyomegaly in two of five males and an increased incidence of tubular regeneration in the females. No treatment-related changes to the kidney were seen in the group given 10 mg of cHPMPC per kg/day.

No treatment-related changes were seen in the esophagus, stomach, large and small intestines, spleen, testes, ovaries, mesenteric lymph nodes, urinary bladder, or injection site of the 150 mg/kg dose group. In addition, no treatment-related changes were seen in the livers of animals of any dose group.

Example 3

Efficacy of HPMPC, cHPMPC and EtHPMPC Against HSV-2 Encephalitis Infection in Mice:

In a preliminary study the antiviral activities of HPMPC, cHPMPC and EtHPMPC against herpes simplex virus type 2 (HSV-2) encephalitis infection in mice were evaluated. In that study the efficacies of the three compounds were very similar using doses of 3, 1, 0.3 and 0.1 mg/kg/day. However, the overall infection was mild since only 50% of placebo-treated mice died. Because of the low mortality rate, this cast some doubt as to the validity of the results with respect to the relative potencies of these compounds. For this reason the present study was conducted whereby the

virus challenge dose was adjusted to cause a more severe infection. This time it appeared that two of the compounds, HPMPC and cHPMPC, were similar in protective activity, and EtHPMPC was poorly active if not inactive.

Compounds: HPMPC, cHPMPC, and EtHPMPC were supplied in dry powder form. They were made up in sterile saline for intraperitoneal (i.p.) administration and stored frozen between treatments. Sterile saline served as the placebo control.

Infection: Swiss Webster female mice (Simonsen Labs, Gilroy CA) weighing approximately 17 grams each at the start of the experiment were infected i.p. with HSV-2 (MS strain) at 2×10^5 plaque forming units (PFU) per mouse. This differs from the preliminary experiment where the mice weighed approximately 20 grams each and received 1×10^5 PFU of virus. This slight adjustment in methodology was important to improve the percentage of mortality in placebo-treated mice.

Treatment: Three hours after virus inoculation, i.p. treatments with compounds and placebo were begun. Treatments were once daily for 5 days.

Parameters used to evaluate the infection: These included death and mean day to death determinations. Deaths were recorded daily for 21 days. The mean day of death calculation took into account only mice that died. Statistical interpretations of survival (Fisher Exact Test) and mean day to death (Mann Whitney U-Test) were made by two-tailed analyses.

Table 3 shows the results of the experiment, indicating that HPMPC was significantly effective in reducing mortality at 1 and 3 mg/kg/day, with lower doses being ineffective. Likewise, cHPMPC caused significant reductions in mortality at 1 and 3 mg/kg/day. EtHPMPC proved to be inactive at the doses tested. Only HPMPC at 0.3 mg/kg/day caused a significant increase in the mean day to death of mice that died, although doses of 1 and 3 mg cHPMPC/kg/day appeared to extend the life span.

Table 3
Effect of Three Antiviral Substances on HSV-2 (MS Strain) Induced Encephalitis in Mice

Compound	Dose[a] (mg/kg/day)	Survivors/ Total (%)	Mean Day to Death
HPMPC	3	10/10 (100)**	>21
HPMPC	1	9/10 (90)**	9.0 ± 0.0
HPMPC	0.3	3/10 (30)	10.9 ± 2.3 *
HPMPC	0.1	3/10 (30)	10.9 ± 4.8
cHPMPC	3	8/10 (80)**	14.0 ± 4.2[b]
cHPMPC	1	8/10 (80)**	18.0 ± 2.8[b]
cHPMPC	0.3	3/10 (30)	11.0 ± 3.1
cHPMPC	0.1	0/10 (0)	9.8 ± 2.3
EtHPMPC	3	3/10 (30)	10.0 ± 2.0
EtHPMPC	1	1/10 (10)	10.0 ± 2.1
EtHPMPC	0.3	1/10 (10)	8.8 ± 1.6
EtHPMPC	0.1	2/10 (20)	8.5 ± 1.4
Placebo	-	3/30 (10)	8.8 ± 1.3

[a] Intraperitoneal treatments were once daily for 5 days starting 3 hours after virus challenge.

[b] Although these values appear to be statistically significant, there are too few data points for analysis.

* $P<0.05$, ** $P<0.001$.

The foregoing examples unexpectedly demonstrate that cHPMPC is up to about 13-fold less toxic than its uncyclized congener, HPMPC, but is quite similar in its antiviral activity. We expect that similar effects will be observed for other HSNAs as well.

Example 4

cHPMPC was synthesized by adding to a stirred suspension of HPMPC (100 g, 0.358 mol) in DMF (2L) N, N'-dicyclohexyl-4-morpholine-carboxamidine (115 g, 0.393 mol). The reaction mixture was stirred for 12 hours at room temperature. This solution was added slowly to a hot pyridine solution (5L, 60°C) of DCC (185 g, 0.895 mol) through an addition

funnel. The reaction mixture was stirred at 100°C for 16 hours, cooled to room temperature and the solvents were removed under reduced pressure. The crude mixture was washed with diethyl ether (3L), dissolved in water (2L) and washed with CH_2Cl_2 (5 x 1L). The aqueous layer was concentrated to 1L volume and acidified to pH-3.5. Upon cooling, cHPMPC crystallized (89 g, ~ 95% pure). The cHPMPC was recrystallized by dissolving in water at pH8 (with 1N NaOH) followed by acidification to pH 3.5 (with 1N HCl). Conversion to the Na^+ form was achieved by dissolving the cHPMPC in methanol (3.3 liters) and adding 0.45 M solution of sodium perchlorate in acetone (15.9 liters). The precipitated cHPMPC monohydrate, monosodium salt, was filtered, washed with acetone (2 liters), and dried under vacuum.

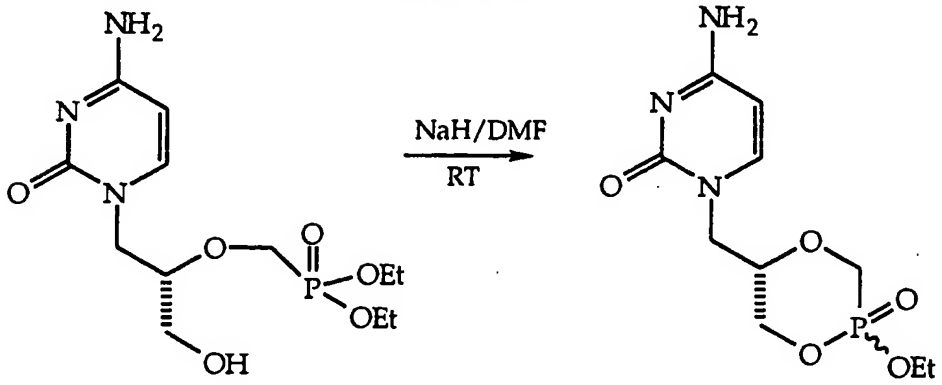
CHN Analysis: cHPMPC monohydrate, monosodium salt. $C_8H_{11}N_3O_5PNa \cdot H_2O$: theory: C31.90 H4.69 N13.96%; found: C32.39 H4.91 N13.95%; 32P-NMR: 9.35(s) (reference H_3PO_4); ^{1}H-NMR: 3.70-4.27 (m, 7H), 4.80 (s,HDO), 6.15 (d, J=7.8, 1H), 7.83 (d, J = 7.8, 1H). ^{13}C NMR (75 MHz, D_2O) d, 169.4 s (4-C), 161.0 s (2-C), 150.2 s (6-C), 98.21 s (5-C), 76.86 d ($J_{P,C}$ = 3.6 Hz, 2'-CH_2), 72.44 d ($J_{P,C}$ = 6.3 Hz, 3'-CH_2), 67.88 d ($J_{P,C}$= 143.0 Hz, P-CH_2), 51.90 s (1'-C).

Example 5

cHPMPU was synthesized by adding thionyl chloride (60 mL, 0.812 mmol, 2.02 eq) dropwise to a suspension of disodium HPMPU (131 mg, 0.404 mmol) in *N,N*-dimethylformamide (1.25 mL) at ambient temperature. The resulting light-yellow solution was stirred for 20 min at ambient temperature and then concentrated to dryness (*in vacuo*, 45 °C). H_2O (2 mL) was added and the resulting solution was concentrated to dryness. Methanol (4 mL) was added and the resulting solution was concentrated to dryness to afford the crude product as a light-yellow solid. Purification by silica flash chromatography (mobile phase: 30% methanol: 70% CH_2Cl_2 gradient to 50% methanol: 50% CH_2Cl_2) afforded pure cHPMPU in 69% yield as a white amorphous solid. ^{1}H NMR (300 MHz, D_2O) d 7.62 d (1H, *J* = 7.1 Hz, C*H*=CH), 5.82 d (1H, *J* = 7.8 Hz, CH=C*H*), 4.30-3.71 m (7H, $CH_2CH(OCH_2P)CH_2OH$), N*H* and O*H* not observed in D_2O. ^{13}C NMR (75 MHz, D_2O) d , 169.6 s (4-C), 155.1 s (2-C), 150.4 s (6-C), 104.2 s

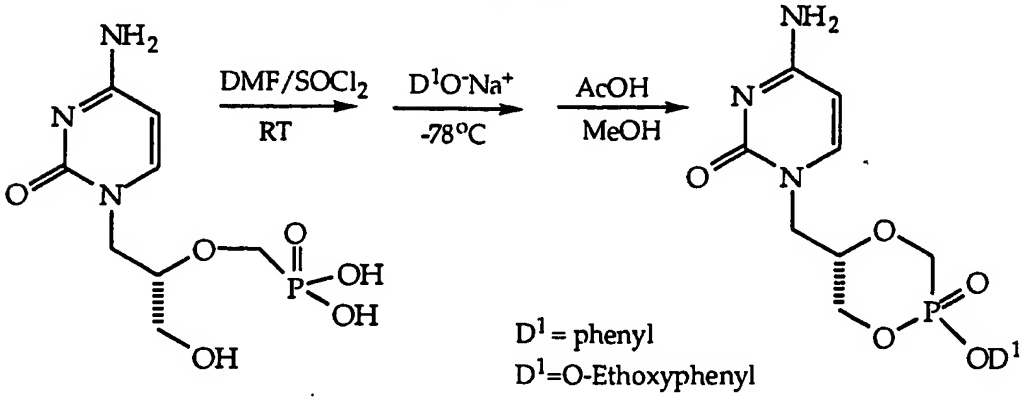
(5-C), 76.71 d ($J_{P,C}$ = 3.6 Hz, 2'-CH_2), 72.30 d ($J_{P,C}$ = 6.2 Hz, 3'-CH_2), 67.90 d ($J_{P,C}$= 142.0 Hz, P-CH_2), 50.71 s (1'-C). ^{31}P NMR (121 MHz, D_2O) d 9.23 s.

Example 6



To a stirred solution of diethyl HPMPC (1.1g) in DMF, NaH (115 mg) was added. After 15 min, the reaction mixture was quenched with acetic acid (1 eq). The solvents were removed under reduced pressure. The crude mixture was dissolved in CH_2Cl_2 and water. The organic layer was washed with NaCl solution and the crude material obtained was purified on a silica gel column (elution with 5%-10% MeOH in CH_2Cl_2) to get cyclic ethyl HPMPC (950 mg) as a diastereomeric mixture (approximately 70%).

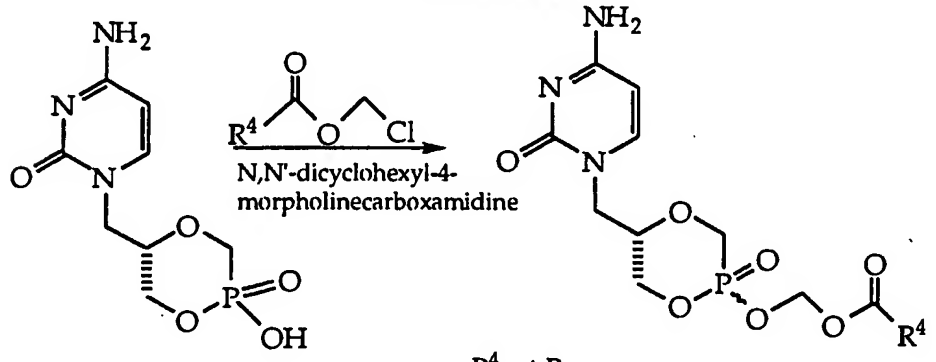
Example 7



To a stirred suspension of HPMPC (2.79 g) in DMF, thionylchloride (2.1 mL) was added dropwise under anhydrous conditions and the mixture was stirred for 1 hr. In another flask, sodium aryloxide (using the

appropriate aryl substituent) was made using the corresponding phenol (8.9 g) and NaH (1.8 g) in 1:1 DMF/THF (50 mL). This solution was cooled to -78°C and the chloridate solution was added dropwise under anhydrous conditions. After 2 hrs, the reaction mixture was quenched with acetic acid (5 eq) and the solvents were evaporated under vacuum. The crude mixture was partitioned between water and CH_2Cl_2. The organic layer was concentrated and the residue was purified on a silica gel column (elution with 5%-10% MeOH in CH_2Cl_2) to get the cyclic aryl compound as a single diastereomer in approximately 60% yield. This method is suitable for all substituted or unsubstituted D' groups, especially aryl, subject of course to conventional protection of labile groups other than amino for which reaction is undesired (amino is protected by reaction with DMF and deprotected with acetic acid and alkanol treatment). This method offers the advantages of producing substantially stereochemically pure product, superior yield and ease of synthesis.

Example 8



R^4 = t-Bu

R^4 = Adamantyl

To a stirred suspension of cHPMPC (1 mmol) was added N,N'-dicyclohexyl-4-morpholinecarboxamidine (2 mmol) followed by the corresponding acyloxymethyl chloride (1.5 mmol). The reaction was stirred for 3 days and the DMF was evaporated under reduced pressure. The crude was purified on a silica gel column (eluted with 5% methanol in methylene chloride) to get the pure cHPMPC derivatives (approximately 30% yield).

Example 9

28-Day Intravenous Toxicity Study of cHPMPC in Rats and Monkeys

This study was designed to characterize the subchronic toxicity of cHPMPC administered in rats. cHPMPC was administered intravenously, daily for 28 consecutive days, to three groups of 12 male and 5 female Sprague-Dawley rats/group at dose levels of 2.5 (Group 2), 10 (Group 3) or 40 (Group 4) mg/kg/day. An additional group (Group 5) of 12 male and 5 female rats was intravenously administered cHPMPC once per week for four consecutive weeks at 70 mg/kg. A concurrent control group (Group 1), comprised of 5 rats/sex received the vehicle only once per day for 28 consecutive days. In all groups of animals, five rats/sex/group were scheduled to be euthanized on Day 29 and 7 males/group (Groups 2-5) were considered to be toxicokinetic animals. All animals were observed twice daily for mortality and moribundity and once daily for obvious indications of a toxic and/or pharmacologic effect. Individual body weight and food consumption measurements were recorded weekly. Blood for clinical pathology evaluations was collected from ten rats/sex not used on study (but from the original shipment of animals) for baseline values. Clinical pathology evaluations (hematology, clinical chemistry and urinalysis) were performed on the study animals prior to termination. On Day 29, 5 rats/sex/group were humanely euthanized and subjected to a complete gross necropsy; each toxicokinetic animal was euthanized following their final PK bleed and discarded without necropsy. Organ weight evaluations and a histomorphologic examination of protocol-specific tissues were performed.

All animals survived until the scheduled termination of the study, with the exception of one Group 2 male found dead on Day 23. There were no apparent cHPMPC-related clinical observations noted in animals from any dose group. In addition, there were no statistically significant differences when mean weekly body weights from treated groups were compared to respective control values. Statistical evaluation of mean food consumption values revealed a significant depression in the Group 4 males on Day 28 as compared to the Group 1 value. Evaluation of mean clinical pathology values revealed the absence of any biologically relevant hematology or urinalysis changes. Significant clinical chemistry changes

included increases in the Group 3 and 4 male and female globulin values, decreases in the Group 4 male and Group 3 and 4 female A/G values, an increase in the Group 4 female cholesterol value, and increases in the Group 4 male calcium and alanine aminotransferase values. There were no statistically significant differences when absolute or relative organ weights were compared between treatment and respective control groups.

At termination, one Group 4 male was noted with pale kidneys which corresponded microscopically to nephropathy and one Group 4 female was noted with a mottled liver which corresponded microscopically to chronic inflammation and endarteritis obliterans. Histomorphologic examination of tissues revealed apparent cHPMPC-related changes in the kidney (mild to moderate nephropathy); femur (moderate to marked osteopathy); bone marrow (focal depletion); and liver (increased severity of chronic inflammation with occasional endarteritis obliterans) of the Group 4 males and/or females.

Based on these data, it may be concluded that there are no apparent effects associated with the daily intravenous administration of 2.5 mg/kg cHPMPC or the once weekly intravenous administration of 70 mg/kg cHPMPC in male and female Sprague-Dawley rats. This is to be compared with a 4 week study in rats where HPMPC was nephrotoxic at 1 mg/kg/day, but not toxic at 0.3 mg/kg/day.

A similar 28-day study with cynomolgus monkeys using doses of 0, 0.5, 2.5 and 10 mg/kg daily or 17.5 mg/kg weekly showed no toxicity at the 0.5 mg/kg/day dose and minimal to mild nephrosis by histopathology at 2.5 mg/kg/day (with pigmentation at the injection site), whereas HPMPC in a 1-month cynomolgus monkey study produced no toxicity at 0.1 mg/kg/day but demonstrated nephrotoxicity at 0.25 mg/kg/day. .

Example 10

Groups of male Sprague-Dawley rats (5 per group) received cHPMPC via oral gavage once daily for five consecutive days to evaluate systemic toxicity. Dose levels were 0, 75, 250, and 500 mg/kg/day administered at a 10 ml/kg dose volume. The control article was sterile deionized water. Approximately 72 hours following the final dose, animals were weighed and bled for clinical pathology. Animals were then euthanized and necropsied. Fourteen tissues were preserved for histopathological evaluation.

No animals died on study. No clinical signs of toxicity nor body weight changes were noted. Clinical pathology changes were limited to slight, non-statistically significant decreases in white blood cell and lymphocyte values at the high dose. Treatment-related histopathological changes were observed only in GI tract tissues and were characterized by inflammation, crypt gland necrosis and/or hyperplasia in the colon and cecum of mid-dose (mild-to-moderate) and high-dose (moderate-to-severe) animals. A mild inflammation of the crypt gland of the duodenum was present in one high-dose animal. A NOEL of 75 mg/kg/day was identified for this study. The bioavailability of cHPMPC is about 20%. Accordingly, a method of this invention comprises orally administering to a subject an antivirally-effective dose of a cHSNA, especially cHPMPC, having structure (VIII) in which A is OH as well as an amidate or ester.

We claim:

1. A method for antiviral therapy comprising administering to a subject an antivirally effective, non-cytotoxic dose of cHSNA which, on a molar basis, is in excess of the maximum non-cytotoxic dose under substantially the same conditions of administration for the corresponding uncyclized HSNA.

2. The method of claim 1 wherein the cHSNA is cHPMPC.

3. The method of claim 2 wherein the dose of cHPMPC exceeds about 3 mg/kg/week or biweek and probenecid is not administered to the subject.

4. The method of claim 2 wherein the dose of cHPMPC exceeds about 5 mg/kg/week or biweek and probenecid is not administered to the subject.

5. The method of claim 2 wherein the dose of cHPMPC exceeds about 10 mg/kg/week or biweek and probenecid is not administered to the subject.

6. The method of claim 2 wherein the dose of cHPMPC exceeds about 15 mg/kg/week or biweek and probenecid is not administered to the subject.

7. The method of claim 2 wherein the subject is infected with CMV.

8. The method of claim 4 wherein hydration is administered to the subject concomitant with cHPMPC.

9. The method of claim 5 wherein hydration is administered to the subject concomitant with cHPMPC.

10. The method of claim 6 wherein hydration is administered to the subject concomitant with cHPMPC.

11. The method of claim 2 wherein fluids are not administered to the subject concomitant with administration of cHPMPC.

12. The method of claim 2 wherein the cHPMPC is administered over a course of therapy in excess of 4 weeks.

13. The method of claim 2 wherein the cHPMPC is administered intravenously once per week or less frequently.

14. The method of claim 1 wherein the cHSNA is administered topically.

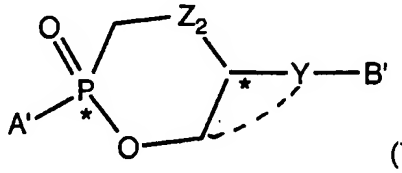
15. The method of claim 2 wherein the dose of cHPMPC exceeds about 1 mg/kg/week or biweek and probenecid is not concomitantly administered to the subject.

16. The method of claim 1 wherein the maximum non-cytotoxic dose is the maximum non-nephrotoxic dose.

17. The method of claim 16 wherein the maximum non-nephrotoxic dose is the maximum dose that fails to produce nephrotoxicity as measured by occurrence of 2+ proteinuria.

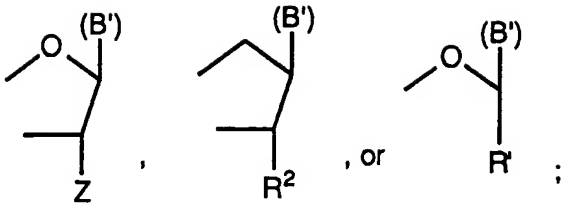
18. The method of claim 16 wherein the maximum non-nephrotoxic dose is the maximum dose that fails to produce nephrotoxicity within about 14 days thereafter, nephrotoxicity being indicated by an increase of >0.5 mg/dl of serum creatinine over the subject's baseline.

19. The method of claim 1 wherein the cHSNA has the structure (VIII)



(VIII)

wherein Z_2 is oxygen or methylene, Y is $-CH_2-$, $-OCH_2-$, -O-



Z is H or C_1-C_6 alkyl; R' is hydrogen, C_1-C_6 alkyl or hydroxyalkyl with 1-6 carbon atoms; R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino; A' is OH or A; A is an amidate or ester; the stereochemistry of the carbon and phosphorus atoms denoted with the asterisks independently are (S), (R) or (RS), the orientation of the Y groups is shown by (B'), and B' is a heterocyclic base.

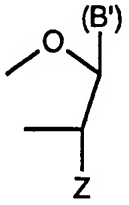
20. The method of claim 19 wherein B' is a 9-purinyl residue selected from guanyl, 3-deazaguanyl, 1-deazaguanyl, 8-azaguanyl, 7-deazaguanyl, adenyl, 3-deazaadenyl, 1-dezazadenyl, 8-azaadenyl, 7-deazaadenyl, 2,6-diaminopurinyl, 2-aminopurinyl, 6-chloro-2-aminopurinyl and 6-thio-2-aminopurinyl.

21. The method of claim 20 wherein B' is adenyl or 2,6-diaminopurinyl.

22. The method of claim 19 wherein B' is a 1-pyrimidinyl residue selected from cytosinyl, 5-halocytosinyl, and 5-(C_1-C_3-alkyl)cytosinyl.

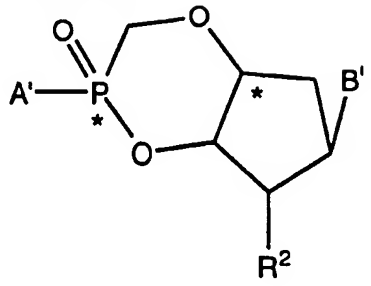
23. The method of claim 19 wherein B' is N-protected cytosinyl.

24. The method of claim 19 wherein Z_2 is methylene, Y is -CH_2-, -OCH_2-, -O-, or



and Z is H.

25. A compound of structure (Ia)



(Ia)

wherein * independently designates (S), (R) or (RS) configuration;

B' is a heterocyclic base;

R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino, or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino;

A' is OH or A; and

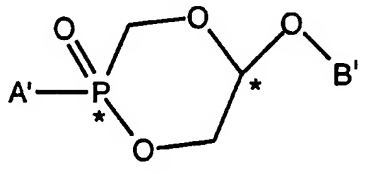
A is an amidate or ester;

and the salts thereof.

26. The compound of claim 25 wherein the carbon atom * chiral center is stereochemically pure as the (S) enantiomer.

27. The compound of claim 24 wherein B' is a 9-purinyl residue selected from guanyl, 3-deazaguanyl, 1-deazaguanyl, 8-azaguanyl, 7-deazaguanyl, adenyl, 3-deazaadenyl, 1-dezazadenyl, 8-azaadenyl, 7-deazaadenyl, 2,6-diaminopurinyl, 2-aminopurinyl, 6-chloro-2-aminopurinyl and 6-thio-2-aminopurinyl, or B' is a 1-pyrimidinyl residue selected from cytosinyl, 5-halocytosinyl, and 5-(C_1-C_3-alkyl)cytosinyl.

28. A compound of structure (Va)



(Va)

wherein * independently designates (S), (R) or (RS) configuration;

A' is OH or A;

A is an amidate or ester;

B' is a heterocyclic base;

and the salts thereof.

29. The compound of claim 28 wherein the carbon atom * chiral center is stereochemically pure as the (S) enantiomer.

30. The compound of claim 30 wherein B' is a 9-purinyl residue selected from guanyl, 3-deazaguanyl, 1-deazaguanyl, 8-azaguanyl, 7-deazaguanyl, adenyl, 3-deazaadenyl, 1-dezazadenyl, 8-azaadenyl, 7-deazaadenyl, 2,6-diaminopurinyl, 2-aminopurinyl, 6-chloro-2-aminopurinyl and 6-thio-2-aminopurinyl, or a B' is a 1-pyrimidinyl residue selected from cytosinyl, 5-halocytosinyl, and 5-(C_1-C_3-alkyl)cytosinyl.

31. A method intended for the preparation of cHSNA intermediates, comprising treating an HSNA with $ClCH{=}N(CH_3)_2Cl$ under conditions suitable to yield a cyclic phosphonylchloridate of the HSNA.

32. The method of claim 31 further comprising reacting the HSNA cyclic phosphonylchloridate with a nucleophile.

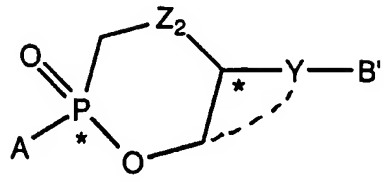
33. The method of claim 31 further comprising subjecting the HSNA cyclic phosphonylchloridate to hydrolysis.

34. The method of claim 31 wherein the HSNA is HPMPC or HPMPA.

35. The method of claim 31 wherein the nucleophile is reacted with the cyclic phosphonylchloridate at a temperature lower than about -20°C.

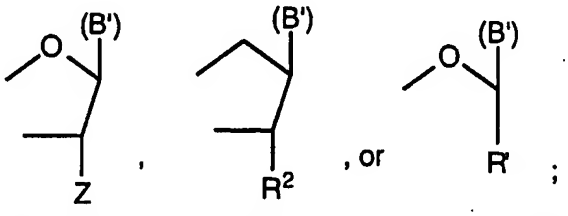
36. A method intended for the synthesis of acyloxymethyl-substituted cHSNA comprising reacting a cHSNA with $R^3C(O)OCH_2Cl$ and N,N' dicyclohexyl-4-morpholinecarboxamidine in molar proportions, respectively, of about 1: about 1-2: about 1-2.

37. An intermediate having the structure (VIIIa)



(VIIIa)

wherein A is an amidate or an ester, Z_2 is oxygen or methylene, Y is $-OCH_2-$, -O-

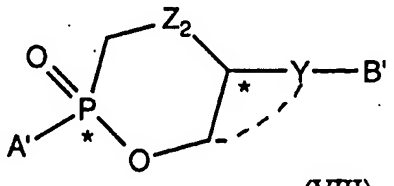


Z is H or C_1-C_6 alkyl; R' is hydrogen, C_1-C_6 alkyl or hydroxyalkyl with 1-6 carbon atoms; R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino; the stereochemistry of the carbon and phosphorus atoms denoted with the asterisks independently is (S), (R) or (RS), the orientation of the Y groups is shown by (B'), and B' is a heterocyclic base provided, however, that (a) A is not a C_1-C_6 alkyl ester or, (b) when Z_2 is oxygen and Y is $-OCH_2-$ then A is not phenyl or substituted phenyl; and the salts thereof.

38. A method comprising orally administering to a subject an antivirally-effective dose of the compound of claim 37.

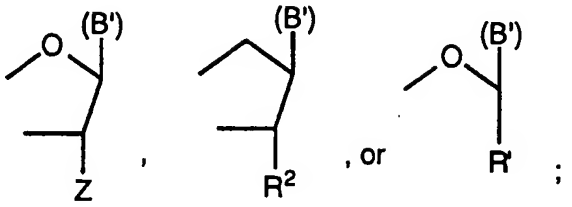
39. The method of claim 38 wherein the intermediate is enriched or resolved at the phosphate atom chiral center.

40. An intermediate having the structure (VIII)

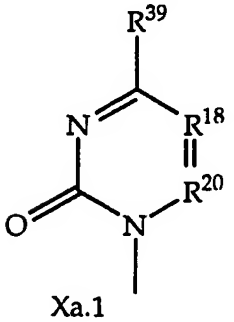


(VIII)

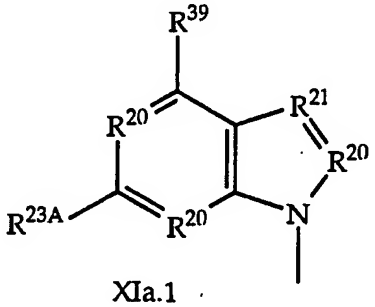
wherein A' is hydroxyl, an amidate or an ester, Z_2 is oxygen or methylene, Y is $-OCH_2-$, -O-



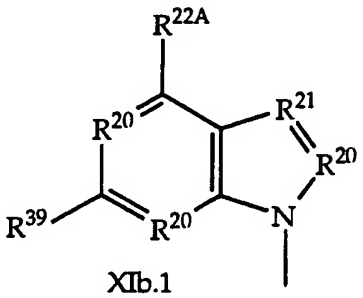
Z is H or C_1-C_6 alkyl; R' is hydrogen, C_1-C_6 alkyl or hydroxyalkyl with 1-6 carbon atoms; R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino; the stereochemistry of the phosphorus and carbon atoms designated by asterisks independently is R, S or RS, the orientation of the Y groups is designated by (B') and B' is a base of the formula (Xa.1), (XIa.1) or (XIb.1)



Xa.1



XIa.1



XIb.1

wherein R^{18} is N, CF, CCl, CBr, CI, CR^{19} or CSR^{19}, COR^{19};

R^{19} is H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2 - C_9 alkynyl, C_1-C_9 alkyl-C_1-C_9 alkoxy, or C_7-C_9 aryl-alkyl unsubstituted or substituted by OH, F, Cl, Br or I;

R^{20} is N or CH;

R^{21} is N, CH, CCN, CCF_3, CC≡CH or $CC(O)NH_2$;

R^{22A} is R^{39} or R^{22}, provided that R^{22} is not amino;

R^{23A} is R^{39} or R^{23}, provided that R^{23} is not amino;

R^{39} is NHR^{40}, $NHC(O)R^{36}$ or $CR^{41}N(R^{38})_2$ wherein R^{36} is C_1-C_{19} alkyl, C_1-C_{19} alkenyl, C_3-C_{10} aryl, adamantoyl, alkylaryl, or C_3-C_{10} aryl substituted with 1 or 2 atoms or groups selected from halogen, methyl, ethyl, methoxy, ethoxy, hydroxy and cyano;

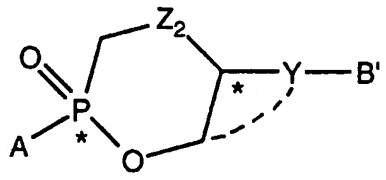
R^{38} is C_1-C_{10} alkyl, or both R^{38} together are 1-morpholino, 1-piperidine or 1-pyrrolidine;

R^{40} is C_1-C_{20} alkyl;
R^{41} is H or CH_3;
and the salts thereof.

41. A method comprising orally administering to a subject an antivirally-effective dose of the compound of claim 40.

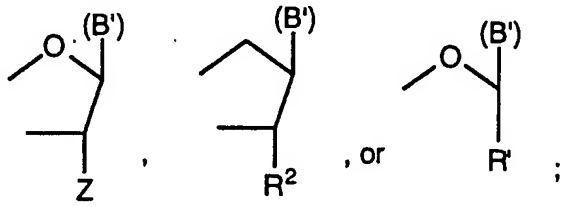
42. The method of claim 41 wherein the intermediate is enriched or resolved at the phosphate atom chiral center.

43. An intermediate having the structure (VIIIa)



(VIIIa)

wherein A is an amidate or an ester; Z_2 is oxygen or methylene; Y is $-OCH_2-$, -O-



Z is H or C_1-C_6 alkyl; R' is hydrogen, C_1-C_6 alkyl or hydroxyalkyl with 1-6 carbon atoms; R^2 is hydrogen, hydroxy, fluorine, chlorine, bromine, amino or an organic substituent having 1-5 carbon atoms and selected from acyloxy, alkoxy, alkylthio, alkylamino or dialkylamino; the orientation of the Y groups is shown by (B'), B' is a heterocyclic base; the stereochemistry of the carbon atom designated by an asterisk is R, S or RS; the intermediate is enriched or resolved at the phosphorus atom chiral center; and the salts thereof.

44. A method comprising orally administering to a subject an antivirally-effective dose of the compound of claim 43.

45. A method for antiviral therapy comprising administering to a subject an antivirally effective, non-cytotoxic dose of cHPMPC in excess of

about 1 mg/kg/week or biweek without concomitant administration of probenecid.

46. The method of claim 45 wherein the subject has CMV retinitis.

47. The method of claim 45 wherein the dose is administered as an equivalent of cHPMPC intermediate.

48. A method comprising orally administering to a subject an antivirally-effective dose of cHPMPC.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/10467

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H19/10 C07H19/20 C07F9/6561 A61K31/70 A61K31/665 A61K31/675

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H C07F A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANTIVIRAL RESEARCH, vol.13, no.6, June 1990, AMSTERDAM pages 295 - 312 A.HOLY ET AL. 'Acyclic Nucleotide Analogues: Synthesis, Antiviral Activity and Inhibitory Effects on Some Cellular and Virus-Encoded Enzymes in Vitro.' cited in the application see page 302; table 1 --- -/--	1,2,7, 14, 19-44,48

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
13 December 1994	-2. 01. 95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Scott, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANTIVIRAL RESEARCH, vol.16, no.1, July 1991, AMSTERDAM pages 1 - 9 R.SNOECK ET AL. 'Antiviral Activity of Anti-cytomegalovirus Agents (HPMPC, HPMPA) Assessed by Flow Cytometric Method and DNA Hybridization Technique.' cited in the application see the whole document	1,2,7, 14, 19-44,48
Y	ANTIVIRAL RESEARCH, vol.13, no.5, May 1990, AMSTERDAM pages 237 - 252 S.B.LI ET AL. 'Activity of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) Against Guinea Pig Cytomegalovirus Infection in Cultured Cells and Guinea Pigs.' cited in the application see the whole document	1,2,7, 14, 19-44,48
Y	WO,A,88 05438 (SRI INTERNATIONAL) 28 July 1988 see the whole document	1,2,7, 14, 19-44,48
Y	EP,A,0 319 228 (BEECHAM GROUP PLC) 7 June 1989 see abstract & US,A,5 247 085 cited in the application	1,2,7, 14, 19-44,48
Y	EP,A,0 398 231 (BRISTOL MYERS SQUIBB COMPANY) 22 November 1990 cited in the application see the whole document	1,2,7, 14, 19-44,48
Y	EP,A,0 369 409 (BRISTOL-MYERS SQUIBB COMPANY) 23 May 1990 cited in the application see abstract	1,2,7, 14, 19-44,48
Y	EP,A,0 494 370 (BRISTOL-MYERS SQUIBB COMPANY) 15 July 1992 see page 1, line 1 - page 6, line 27 see claim 1 & US,A,5 208 221 cited in the application	1,2,7, 14, 19-44,48

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/10467

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOLECULAR PHARMACOLOGY, vol.41, no.1, January 1992 pages 197 - 202 HO ET AL. 'Intracellular Metabolism of the Antiherpes Agent (S)-1-(3-Hydroxy-2-(phosphonylmethoxy)propyl)cytosine.' cited in the application see the whole document	1,2,7, 14, 19-44,48
P,Y	WO,A,94 03466 (SRI INTERNATIONAL) 17 February 1994 cited in the application see the whole document	1,2,7, 14, 19-44,48
P,Y	WO,A,94 03467 (INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY OF THE ACADEMY OF :...) 17 February 1994 see the whole document	1,2,7, 14, 19-44,48
A	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol.94, no.14, 12 July 1972, GASTON, PA US pages 5070 - 5076 M.SUNDARALINGAM ET AL. 'Stereochemistry of Nucleic Acids and Their Constituents. XXVII. The Crystal Structure of 5'-Methyleneadenosine 3',5'-Cyclic Monophosphate Monohydrate, a Biologically Active Analog of the Secondary Hormonal Messenger Cyclic Adenosine 3',5'-Monophosphate. Conformational "Rigidity" of the Furanose ....' see page 5070; figures 1,AND,2	19,20

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/ 10467

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Please see attached sheet ./.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

The claims not searched are not permitted under Articles 10(2) and 34(2) PCT, and are furthermore not able to be reformulated in an acceptable manner (as some of the others e.g. 1, 2, etc. are) since they refer to dosages and the timings of said dosages.

Claims not searched : 3-6,8-13,15-18,45-47
Claims searched completely : 1,2,7,14,19-44,48

Remark: Although claims 1,2,7,14,19-24,38,39,41,42 and 48 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

information on patent family members

International application No.
PCT/US 94/10467

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